

Steven M. Ruben
Appl. No. 10/662,429

Department MOL. Bio.
Subject 9/15/95 - 12/29/95
Name ANN KIM # 11
Address _____

National® Brand

Computation Notebook

11 3/4" x 9 1/4", 4 x 4 Quad., 75 Sheets

43-648



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Chicopee, MA 01022

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Ruben EXHIBIT #94

Department Mol. Bio.
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Ruben EXHIBIT 2094
Ruben v. Wiley et al.
Interference No. 105,077
RX 2094

(See Pg 152 Book #10 → Lab Notebook #

345)

9/15/95

Wash Blot 1X PBS

5 min at RT w/ Shaking
Add 2° Ab at 1:2000
in PBS

incubate at RT for 1 hour with
Shaking

Rinse in 1X PBS

Wash 5 min at RT with 1X PBS

Rinse in 50 mM $\text{Na}_2\text{H}_2\text{PO}_4$ pH 7

5 min at RT

Add Substrate:

12.5 ml 50 mM $\text{Na}_2\text{H}_2\text{PO}_4$ pH 7

25 mg β -NADH

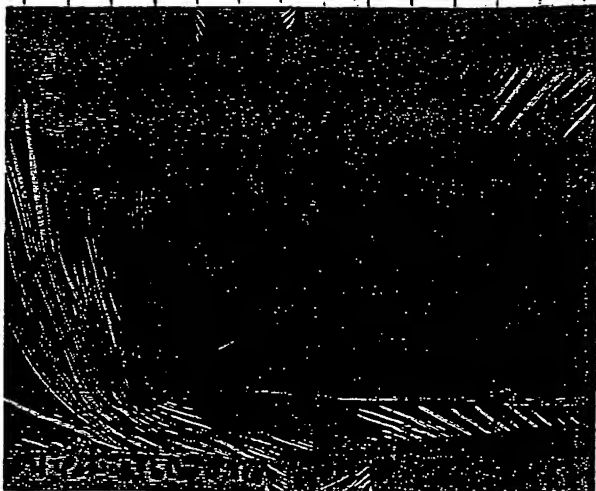
25.0 μ l phenol

8.5 μ l 30% H_2O_2

0.375 ml NBT (10 mg/ml)

incubate at RT until color develops

Stop Reaction with dH₂O



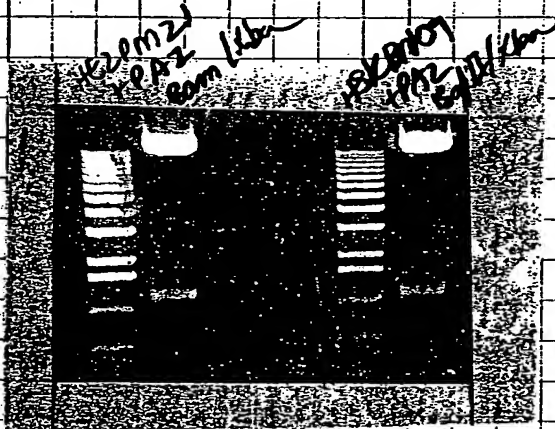
freez Os Cells
Split 1 flask 1:40

9/22/95

Run 10ul on gel w/ 1kb ladder

looks good

Submit for Sequencing



HE2PM21+PA2

RP01
RP15
RP16
RP18
RP81
FP02
FP11
FP14
FP17
FP80

H5K6N07+PA2

RP01
RP07
RP08
RP81
FP04
FP06
FP80

Heidi
9/24/95

9/25/95

Transfections of Lac Z / β -gal
in 100 μ g/ml & 500 μ g/ml
RDEAE - O Dextran

Remove Supernatant from Cells
Wash Cells 1x PBS 5min
at RT

Fix Cells 3.7% Formaldehyde in PBS
Incubate at RT 30min

Remove Fix

Wash Cells 2x PBS 5min
at Room Temp

9/25/95

Submit HE2PM21 + PAZ
to protein Expression.

Set-up Cells - COS Cells
for transfections
6 Well plates
 1.5×10^5 cells / well

Clean-up DNA for transfections

HTPANO8SD4 pCDNA
pCDNA 3' HA
pCDNA 5' HA

HT45B02 pCDNA
pCDNA 3' HA
pCDNA 5' HA

2X Phenol:Sevag (1:24, 1AA:chloroform)
2X chloroform

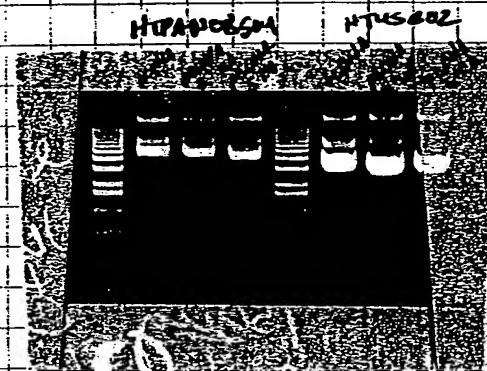
2X EtOH ppt
Wash ppt, ethanol - Let pellet Dry
Resuspend pellets in 100ul TE

9/26/95

Dilute DNA 1:200 in H₂O
Read OD₂₆₀/280.

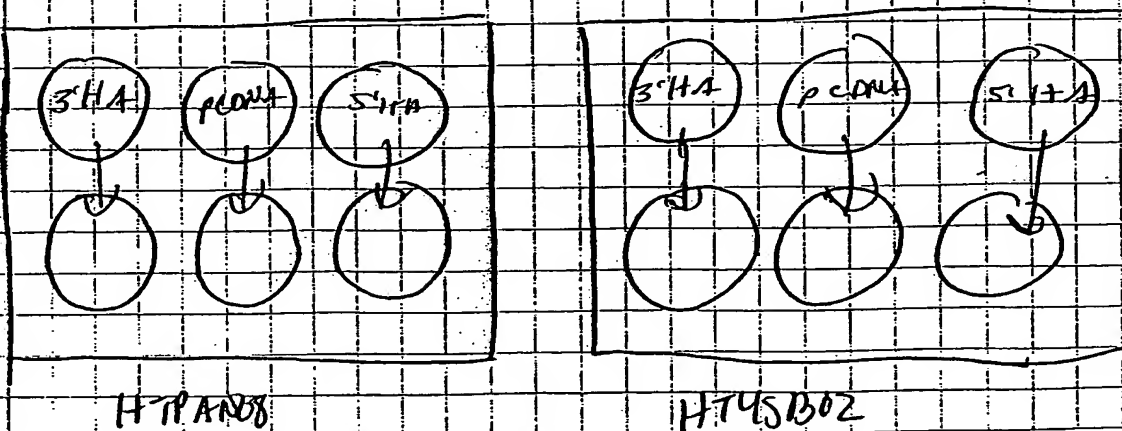
	260.0 nm	280.0 nm		260.0 nm	280.0 nm
HTPAB08 pCDNA	0.0460	0.0311	1.4833	0.8742	0.46 ug/ml
HTPAB08 3'HA	0.0400	0.0277	1.4586	0.8865	0.40
HTPAB08 5'HA	0.0400	0.0300	1.3024	0.7678	0.5
HTAB08HT4502	0.0400	0.0350	1.5920	0.6281	0.88
HT4502 3'HA	0.0400	0.0300	1.4943	0.8692	0.91
HT4502 5'HA	0.0400	0.0450	1.5087	0.8626	0.68

Set up transfection
Run 1ul on gel with
1 kb ladder



Set up transfections:

Use 100 ug/ml DEAE
Dextran
looks like it works the
Best from Lac Z - Beta Gal Test



9/26/95

HT4SBO2	pcDNA	0.88 $\mu\text{g}/\mu\text{l}$	^{5 μg} 5.68 μl
	pcDNA 3' HA	0.91 $\mu\text{g}/\mu\text{l}$	5.51 μl
	pcDNA 5' HA	0.68 $\mu\text{g}/\mu\text{l}$	7.35 μl

HTPANDOS04	pcDNA	0.46 $\mu\text{g}/\mu\text{l}$	10.9 μl
	pcDNA 3' HA	0.4 $\mu\text{g}/\mu\text{l}$	12.5 μl
	pcDNA 5'	0.5 $\mu\text{g}/\mu\text{l}$	10.0 μl

make 15 ml transfection Cocktail

1.5 ml Nucleofectin
 150 μl DEAE Dextran
 150 μg Chloroquine 5 mg/ml

Mix well

Add 1 ml transfection Cocktail

to DNA
 Mix well

Add to well

incubate 37°C / 5% CO₂ for
 2 hrs.

Shock Cells

10% DMSO in cDMEM
 2 min at RT

Remove Supernatant

9/28/95

TRANSFECTIONS -

HT4SBO2 + pCDNA / 5'HA / 3'HA

HTPANOR + pCDNA / 5'HA / 3'HA

Cells have been incubating at 37°C
 $5\% \text{CO}_2$ for 48 hrs \checkmark

Remove media

Wash Cells 1X PBS 5min

Fix cells w/ 3% Paraformaldehyde
in PBS.

1.5g paraformaldehyde

45ml H_2O 12.5ml 10N NaOH Heat $40-50^{\circ}\text{C}$ Add 5ml 10X PBS .

Cool to RT

Fix for 30-35min at RT

Wash Cells 10min RT with
PBS + 10mM Glycine Incubate 5min - Time is critical -
with 1% Triton X-100 in PBS.

Wash Cells 5min RT with

PBS + 10mM Glycine Incubate Cells $1\frac{1}{2}$ hrs RT with
PBS + 25mM Glycine .

Block Cells in Antibody Binding Buffer:

 $20\text{mM NaH}_2\text{PO}_4$ pH 7.0 0.5M NaCl 0.05% Tween-20 1% BSAWith 2% Goat Serum for 2 hrs at
Room Temp

9/28/95

Remove Blocking Buffer
 Add 1° Antibody into Antibody
 Binding Buffer + 2% Goat
 Serum
 Use HA TAG - SCP-12CA5-J
 at purified Ab.
 at 1:1000 dilution
 Incubate at 4°C O/N

ENTERED Project information for:

HSRBN09
 HT4SBOZ
 HE8SH43

~~HE2PM21~~ → HDAAHT682
 into IRIS!

Split Cos Cells 1:40 into
 T-75 flask

9/29/95

Remove 1° Ab
 Wash Cells 2x - 10 min RT
 with 10 mM Glycine in PBS
 Remove Wash
 Add 2° Ab 1:2500 RITC conjugated
 Antibody in Ab Binding Buffer + 2%
 Serum
 Incubate ~~30~~ RT 1 hr w/ alum foil
 Covered

9/29/95

Aspirate off 2° Ab
 Rinse cells 1 X 10mM Glycine + PBS
 Aspirate off Rinse
 Wash cells 2 X - 2m1
 10mM Glycine + PBS
 Aspirate off Wash
 Add 2 drops of photo mount
 and cover w/ glass cover slip
 View under fluorescent microscope
 Keep covered in Alum. foil at
 4°C.

Submit -
 HE2PM21 + PAZ
 HSKBNO9 + PAZ
 HE2PM21.
 for Sequencing

10/2/95

Split Cos Cells
 1:40 into T-75 flask
 and plate out 2 6-well plates
 for transfections
 Seed plates at $\sim 1 \times 10^5$ cells/well
 Incubate 37°C 5% CO₂ O/N

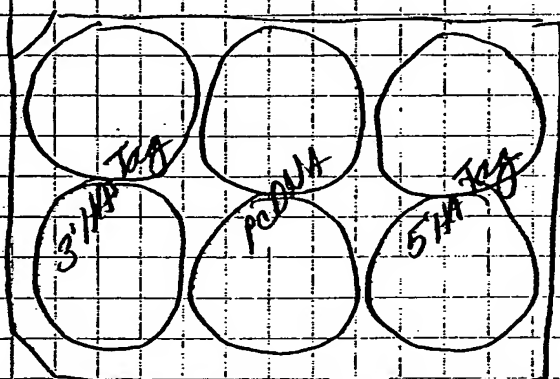
PCR New
 HE2PM21 - POREO insert
 New 5' WCO I primers made

0/3/95

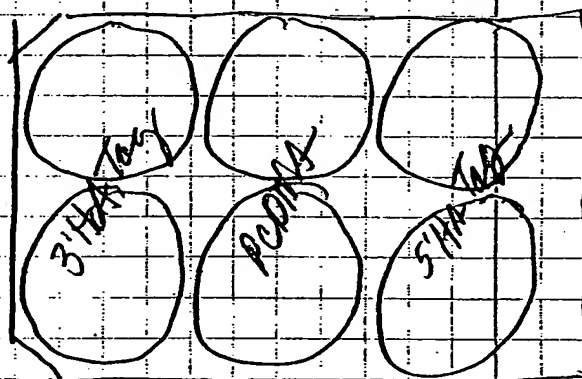
Transfections:

		conc μg/μl	5' cap
HT4SBO2	pCDNA	0.88	5.68 μl
	pCDNA 5'HA	0.68	7.35 μl
	pCDNA 3'HA	0.91	5.5 μl
HTPAN08 5'bpATG	pCDNA	0.46	10.9 μl
	pCDNA 5'HA	0.5	10 μl
	pCDNA 3'HA	0.4	12.5 μl

Set up Duplicates of each.



HT4SBO2



HTPAN08 5'bpATG

Make 15 ml's Transfection Cocktail

1.5 ml Neutravidin
 150 μl DEAE-Dextran (10 mg/ml)
 150 μl Chloroquine (5 mg/ml)
 mix well.

Add 1 ml to 5 μg DNA & Pipet well

Add to well

incubate 37°C 5% CO₂ for 2 hrs.

Remove Transfection Cocktail from wells

10/4/95

Inoculate 4ml LB + Amp / Kan:

HTPB411 + PQE60 - 3-4
 HMSAF22 + PQE60 - #A
 HTPAN08 51bp ATG + PD10 - D9
 " " " + " - D5
 " " " + " C7
 " " " + " A2

Inoculate 4ml LB + ~~Amp~~ Kan:
 M15 up 5 cells

Inoculate from frozen glycerol stocks.

Incubate 37°C 5 hours -
 till OD₆₀₀ 0.4-0.6

TO 1000ul LB + Amp Kan
 + transfer 100ul of culture
 TO remaining culture add 100mM
 IPTG TO 2mM - 80ul
 Incubate 4 hours
 Store at RT O/N.

Sterilize
 10/4/95

10/5/95

Run 2 15 well 12.5% Gels
 with induced and uninduced
 cultures.

Spin @ 500ul Culture 2 min
 Aspirate off supernatant -
 Resuspend pellet in 100ul H₂O

10/5/95

Add 100 μ l 2x Dissociation Buffer
 Mix well
 Heat Samples 100°C 5 min
 Spin 45 min
 Load 12.5 μ l onto gels

lane Sample

- | | | |
|----|----------------|-----------------------------|
| 1 | Uninduced | > M15 cells |
| 2 | Induced | |
| 3 | U | > HTPB411 + PQE60 3-4 |
| 4 | cl | |
| 5 | U | > HMSAF22 + PQE #19 |
| 6 | cl | |
| 7 | U | > HTPAN08 51bpATG + PD10 D7 |
| 8 | cl | |
| 9 | Rainbow Marker | |
| 10 | U | > HTPAN08 51bpATG + PD10 D7 |
| 11 | cl | |
| 12 | U | > " " " + " C7 |
| 13 | cl | |
| 14 | U | > " " " + " A2 |
| 15 | cl | |

Run Gel - 150V 1 hour
 10 / Gel - Set up wet transfer.

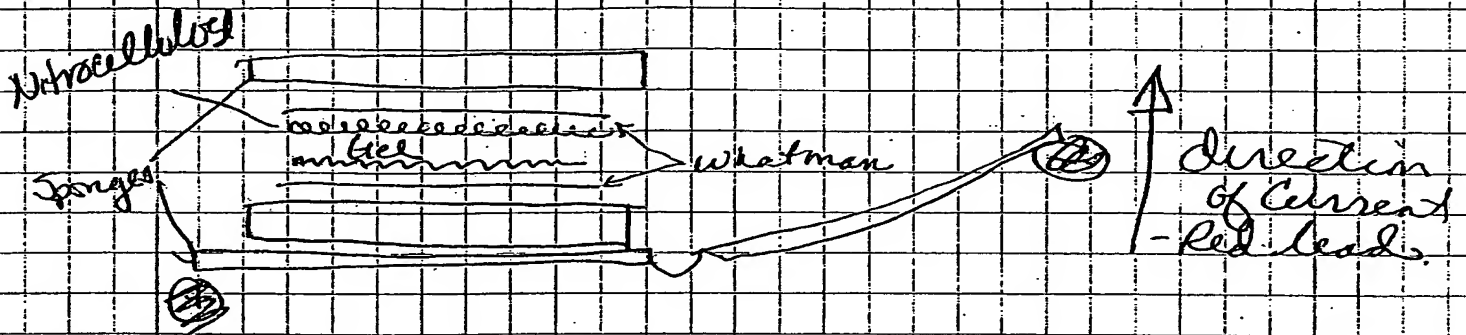
Transfer Buffer:

25 mM Tris pH 8.3
 192 mM Glycine
 20% Methanol

-Cold

Setup Gel Sandwich in Support.

10/5/95



Run 100 V 1 hour.

Block Blot in
Western Blocking Buffer:
3% BSA (V)
0.02% Na-Azide
in PBS

Incubate at RT w/ Shaking
2 hrs

Pour off Blocking Buffer.
Add 1° Ab in Western Blocking
Buffer at 1:100 dilution.

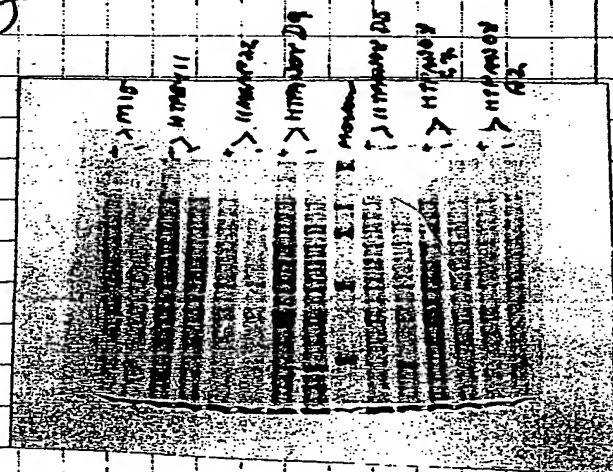
Use Rabbit Antibody
#11941 - large bleed

Incubate at Room Temp Overnight
w/ Shaking

With other gel
Stain
DESTAIN.

Take picture
of gel

Inoculate 50 ml
1 B Amp Kan with
HTPA1008 D9



10/5/95

HE2PM21 + PQE60-

Transform into M15 sup 4 cell

Thaw Chemically Competent Cells
on iceTo 100 μ l of thawed cells add
10 μ l of ligation
mixture on ice for 1 hour
Heat Shock 42°C 1 min

Place in ice

Add 400 μ l LBIncubate 37°C 1 hour

Plate onto LB-amp/Kan plates

Incubate 37°C overnight

Transfections

H1PAN08 / H1USB02

Remove Complete DMEM

Wash Cells 1X PBS 5 min

Remove Wash

Fix Cells 3% para-formaldehyde
in PBS

50 ml

15 mg Paraformaldehyde in
45 ml PBS

Add 12.5 ml 10N NaOH

Heat $40-50^{\circ}\text{C}$ to dissolve

Cool to Room Temp

Fix Cells in 2 ml Paraformaldehyde
for 30 min at RT w/ shaking

Remove fixer

Wash Cells 10 mM Glycine 4X PBS
5 min at RT w/ shaking

10/5/95

Remove Wash
 To 1 set - add 1% Triton X100 in
 1xPBS - mix well
 Incubate 5min at RT
 To other set wash with 10mM Glycine
 + 1xPBS
 Remove Triton X100 solution
 Wash cell 10mM Glycine + 1xPBS - 5min
 Remove Buffers
 Wash cells 10mM Glycine + 1xPBS
 5min w/ shaking
 Remove Wash
 Incubate cells 25mM Glycine
 + 1xPBS at Room temp 1 1/2 hrs.
 with shaking

Block Cells in Antibody Binding
 Buffer + 5% Goat Serum
 20mM $\text{Na}_2\text{H}_2\text{PO}_4$ pH 7.0
 0.5M NaCl
 0.05% Tween 20
 1% BSA

Incubate at Room Temp 2 hrs
 with shaking

Remove Blocking Buffer
 Add 1 μ Antibody into Antibody
 Binding Buffer
 use HA-Tagged purified Ab
 1:1000 dilution
 Incubate 40 $^{\circ}$ C O/N with
 shaking

10/6/95

Transfections - HT4SBO2 / HTPA208

Remove primary antibody

10/6/95

Wash Cells 2X in 1X PBS + 10mM Glycine
 5min at Room Temp
 Remove Wash
 Add 2° Ab - at 1:2000 Dilution
 RITC conjugated Ab
 in Antibody Binding buffer +
 2% Goat Serum
 Incubate at RT w/ Shaking
 for 2 hours Covered w/Aluminum foil
 Remove 2° Ab
 Rinse Cells 2X in 1X PBS + 10mM Glycine
 at Room Temp 5min
 Remove all of liquid
 Add 1 large drop of photomount
 Cover with cover slip
 Store 4°C Covered w/Aluminum foil

HTRAND8 Western

Pour off 1° Ab
 Rinse off filters in 1X PBS
 Wash filters in 1X PBS 5min at RT
 Add 2° Ab - Anti Rabbit Peroxidase
 - Dilute 1:2000 in 1X PBS
 Incubate at Room Temp with Shaking
 2 hours
 Rinse filters 1X PBS
 Wash filters 1X PBS - 5min at RT
 Rinse filters in 50mM NaH₂PO₄ pH 7
 Wash filters 50mM NaH₂PO₄ pH 7 for 5min at RT w/ shaking

10/6/95

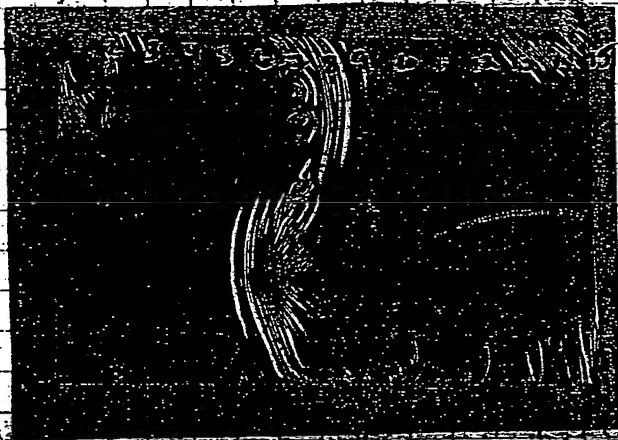
HTPAN08 Western

Add Substrate:

12.5 ml 50 mM NaH_2PO_4 pH 7
 25 mg β -NADH
 25 ml phenol
 8.5 ml H_2O_2
 37.5 ml NBT- Nitro blue Tetrazolium
 10 mg/ml

Incubate at RT until color change is seen and developed to desired Darkness.

Stop Reaction with distilled H_2O .
Dry on filter paper



- | | | |
|----|---|----------------|
| 1 | + |] m15 |
| 2 | - | |
| 3 | + |] HTB411 |
| 4 | - | |
| 5 | + |] HNSAF22 |
| 6 | - | |
| 7 | + |] HT PAN08 D9 |
| 8 | - | |
| 9 | | Rainbow Marker |
| 10 | + |] HT PAN08 D5 |
| 11 | - | |
| 12 | + |] HT PAN08 C7 |
| 13 | - | |
| 14 | + |] HT PAN08 AZ |
| 15 | - | |

HT PAN08 + PD10 #0 D9
Induction

Inoculate 1000 ml LB + Amp/Kan
with 30 ml of over night Culture

10/6/95

HIPANOS PD10 D9-Induction
 Incubate 37°C w/aeration
 till $\text{OD}_{600} = 0.4 - 0.6$ - incubated
 2 1/2 hours
 Add 100mM IPTG to 2mM -
 $6.30\text{mL} \rightarrow 12.6\text{mL}$
 Incubate 4 hours w/aeration
 at 37°C
 Spin Cultures 3K 30 min
 Pour off Supernatant
 Resuspend pellet @ total of
 70mL 6M GnHCl $\text{pH } 8$
 Store 4°C over the weekend

HE2PM21 + POE60

Inoculate 200ul $1\text{B} + \text{Amp}/\text{kan}$
 in 96 well dish.
 Inoculate with Colonies -
 72 of HE2PM21 + POE60 and
 6 of POE60 kan/No
 Incubate 37°C at Room Temp
 over the weekend

10/9/95

HE2PM21 + POE60 PCR.

Check Cultures for Inserts

10/9/95

HTPAU08 PD10 D9 Induction

Spin Cells in 6M GnHCl 8K
30 minTransfer Supernatant to fresh
tube
Store On ice

Prepare Ni-NTA Column

To Column Add 5ml Resin Slurry

Wash with 30ml di H₂OCharge Column by adding 30ml
0.10M NiSO₄Wash with 30ml di H₂O

Equilibrate 30ml 6M GnHCl pH 8

Apply Supernatant

Allow to flow - Collect as flow
through

Wash 50ml 6M GnHCl pH 8

- collect pH 8

Wash 50ml 6M GnHCl pH 6

- collect pH 6

Elute Protein - 8ml 6M GnHCl pH 5

- collect pH 5

Strip Column - 50ml 6M GnHCl pH 2

- collect pH 2

Store at 4°C till tomorrow -

Run on Gel

10/10/95

HE2PM21 7PQ6 Co Inductions

Heat Samples 100°C 5min

Spin 2 min

Load 12.5ul per well

10/10/95

HTPAN08 PD10 D9.

Run samples on gel

H₂O 4.50 μ l
 Samples 50 μ l
 0.15% NaDoc 35 μ l
 50% TCA 50 μ l

Mix well

Spin 10 min

Remove Supernatant

Resuspend pellet in 10 μ l 0.2N NaOHAdd 10 μ l 2X dissociation Buffer

Mix well

Heat 100°C

5 min

Run 20 μ l on 12.5% SDS-PAGE

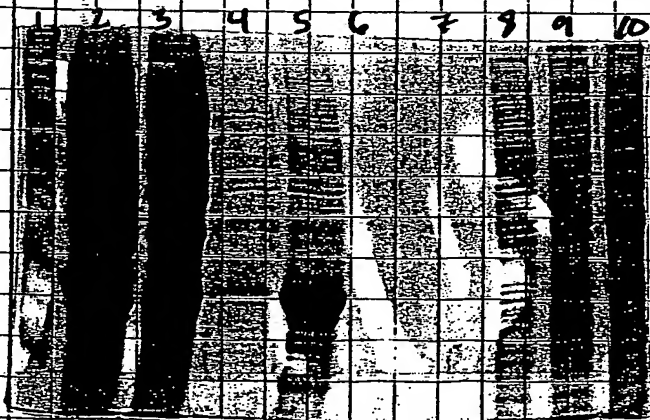
Stacking gel

Run 150V 1 hour

STAIN

DESTAIN

Dry on Bio Rad Air Gel Dryer



- 1- Marker
- 2- ~~HTPAN08~~ Total Crude Extract
- 3- Flow
- 4- pH 8
- 5- pH 6
- 6- pH 5
- 7- pH 2

HTPAN08 PD10 D9

- 8- UN
- 9- 54
- 10- 55

HEUPM21
HPD10

10/10/95

Looks like there is still a lot of protein left in the flow -
 Rappley to Column
 - pH was off - Most of Protein came off of Column.

10/11/95

#

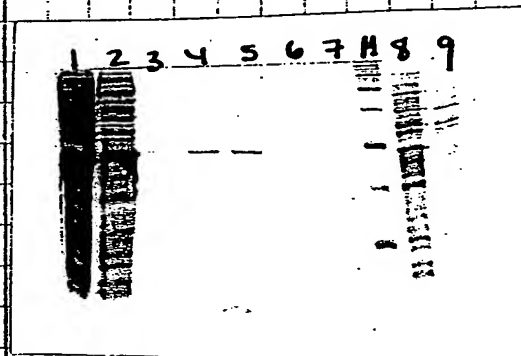
Regenerate Column.

30 ml H₂O
 30 ml D.I.M.W. Soy
 30 ml H₂O
 30 ml 0.1M HCl pH 8.

Add pH 8 to Flow from 10/10/95

Add 0.1M HCl pH 8
 center protein off of Column - See pg 42
 10/9/95

Run 50ul on gel with 1Kb ladder



1 - Crede Extract

2 - Flow

3 - pH 8

4 - pH 6

5 - pH 5

6 - pH 2

7 - pH 5 from 10/9/95

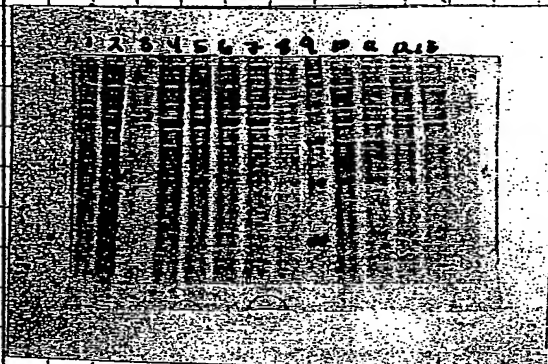
M - Rainbow Marker

8 - HEPANOS + PDIO

9 - D9, D5

10/11/95

Run Remaining Samples of HE2PM21 + PQE60
on gel with Rainbow Marker
Stain DeStain



- 1 HE2PM21 + PQE60 5p
 - 2 57
 - 3 58
 - 4 59
 - 5 60
 - 6 61
 - 7 62
 - 8 UN induced
 - 9 Rainbow Marker
 - 10 D9
 - 11 D5
 - 12 C7
 - 13 A2
- HE2PM21
from
10/5/95

Inoculate 1 BT ~~20~~ + 15 mM MgCl₂
with M15 rep4 & XL-1 Blue

10/12/95

Make Chemically Competent XL-1 Blue
and M15 rep4

To 2 - 2L flasks with 300 ml
1 BT + 15 mM MgCl₂
Add 3.5 ml of O/N culture
Incubate 37°C w/ aeration 2 - 2 1/2
hours until OD₆₀₀ = 0.4 to 0.6.
Transfer Culture to Sterile Centrifuge
Bottles. Cool on ice 30 min.
Spin 5K for 30 min at 4°C.
Decant Supernatant
Resuspend pellets in Solution A
ice cold for every 500 ml resuspend
in 300 ml

10/12/95

Solution A

50 mM $MnCl_2 \cdot 4H_2O$ 50 mM $CaCl_2$

10 mM MES pH 6.3

- make Day ahead - store $4^\circ C$.

let Cell suspension sit on ice

20 min

Spin Cells 5K 30 min

Resuspend cells in ice cold Solution

A + ~~1000~~ 15% Glycerol

Resuspend in 3ml for every 50ml

Starting volume - ~84ml

Aliquot 500ul into Sterile tubes.

and then freeze on dry ice.

Store at $-70^\circ C$

Reapply HIPANOS 51bp ATG PD10 DA

to column.

Add more resin to column bed.

Regenerate Column.

- See protocol pg 42 (10/9/95)

Run 50ul on gel.

50 ul Sample

75 ul 50% TCA

50 ul 0.15% NaDOC.

450 ul H_2O

Mix well

Resuspend

Add 10 ul

Heat $100^\circ C$

Spin 10 min

pellet 10 ul

0.2N NaOH

2X Association Buffer

5 min

10/12/95

Quick Spin 1 min
 Run on 15% Acrylamide
 Stacking gel 150V 1 1/2 hrs
 Stain 0.38 min 34°C
 Destain O/N at RT.

HELM21 + PEG60

B. Mini preps

Spin 1.5 ml Culture 2 min
 Remove Supernatant
 Resuspend pellet in 600 µl

STE1 + RNase + Hepoxyme
 Boil 5 min

Spin 10 min

Remove Pellet

Add 600 µl 13% PEG/1M NaCl

Mix well

Spin 10 min

Remove supernatant

Wash pellet 1000 µl 70% Ethanol

Spin 5 min

Remove Supernatant

Allow Pellet to air Dry

Resuspend pellet 50 µl TE

Run 1 µl on gel with 1 Kb ladder

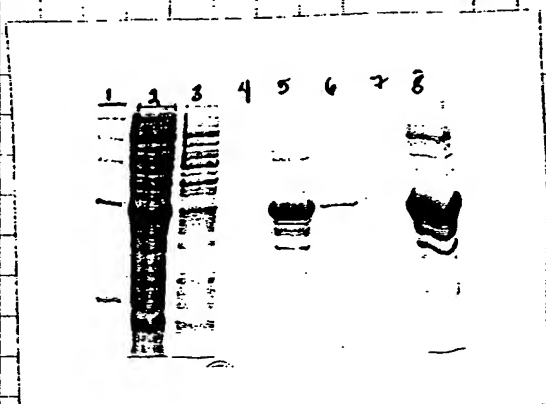


Logho left
 NO DNA

May need to
 re-do

10/13/95

H-TRANOX 5bp ATG + PD10 DA



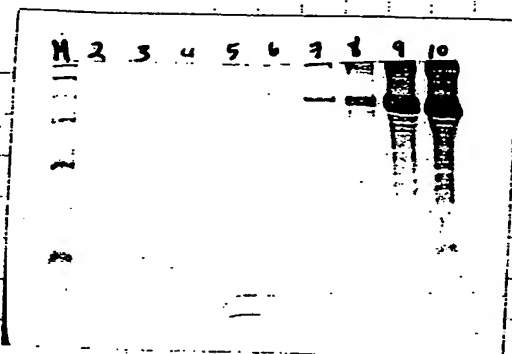
1 Rainbow Marker
 2 Crude Extract
 3 flow
 4 pH 8
 5 pH 6
 6 pH 5
 7 pH 2
 8 pH 5 - from 10/11/95

10/18/95

Run Gel -
 Quantitative gel of BSA 10mg/ml

2 10ng
 3 50ng
 4 100ng
 5 500ng
 6 1ug
 7 5ug
 8 10ug
 9 100ug
 10 500ug

Run on 15% PA Stacking
 gel with Rainbow
 marker
 150 V 1 1/2 hrs.
 STAIN
 DESTAIN



10/16/95

Run Preparative Gel of
 HTPANOS 5bp ATG + P870 D9
 pH6.

200ul	pH6.
150ul	50% TCA
100ul	0.15% N ₂ DOC
800ul	H ₂ O

2X

Mix well

Spin 10 min

Remove Supernatant

Resuspend pellet in 400ul of DQN N200

Add 400ul 2X dissociation Buffer

Run on @ 15% Preparative stacking
gel100V 1 1/2 hrs
Cut off part of gel w/ marker and
some of gel

STAIN

DESTAIN.

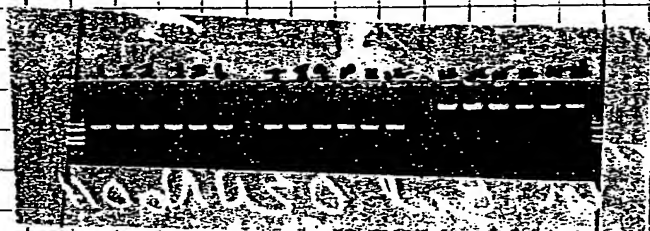
Cut out band at appropriate size

Store Gel slabs at
 4°C in 15ml Conical
 Tube



10/25/95

Incubate 37°C 10 hrs
Run 1 µl on gel with 1 Kb ladder



Add 150 µl TE
Add 20 µl 3M NaAcet
Add 450 µl 100%
Ethanol

Store -20°C

1	BamHI / BglII	} pG6d
2	BamHI / NcoI	
3	BglII / Bam	
4	BglII / NcoI	
5	NcoI / BamHI	
6	NcoI / BglII	

7	BamHI / BglII	} pG67
8	BamHI / SphI	
9	BglII / BamHI	
10	BglII / SphI	
11	SphI / BamHI	
12	SphI / BglII	

13	KpnI / BamHI / BamHI	} pA2
14	BamHI / BglII	
15	BamHI / KpnI	
16	Asp718 / BamHI	
17	BglII / BamHI	
18	KpnI / BamHI	

Run Gel of HIPANOS Ecoli purified proteins
to determine concentrations

Samples: HIPANOS 185bp ATG 2/15
51bp ATG 2nd Unind. Elect 5/20
51bp ATG pH5 10/11
51bp ATG pH5 10/12
51bp ATG pH6 10/12

Use BSA - 10 µg/ml as standard.

10/25/95

Run 10 μ l of HTP AD Sample

500 μ l H_2O
 50 μ l 0.15% NaDOC
 75 μ l 50% TCA
 10 μ l Sample

Mix well

Aspirate off Supernatant

Resuspend pellet in 8 μ l 0.2N NaOHAdd 8 μ l 2x Dissociation Buffer

Heat 100°C 5 min

Run entire amount on 10% Acrylamide gel

BSA -

500 ng
 750 ng
 1 μ g
 2 μ g
 3 μ g
 4 μ g
 5 μ g
 10 μ g
 100 μ g

To all samples
 dilute to desired
 amt. Add 10 μ l 2x
 dissociation Buffer
 Heat 100°C 5 min
 Run entire amt on
 gel with marker

Run 1SDV 1.3 hrs

Stain 37°C 1 1/2 hr

DESTAIN over night at RT

Send 100 μ g Proteins to Rick Ling
 at Georgetown
 2 Boxes of the
 TGF α

10/26/15

Take Picture of HTPAN08 Gel



1	185bp	2/15	}	HTPANCE
2	51bp	2nd elut		
3	51bp	pH 5 10/11		
4	51bp	pH 5 10/12		
5	51bp	pH 6 10/12		
6	500 ng	}	BSA	
7	750 ng			
8	1 ug			
9	2 ug			
10	3 ug			
11	4 ug			
12	5 ug			
13	10 ug			
14	100 ug			

HTPAN08	185bp	~ 600	→ 60 ng/ul
HTPAN08	51bp	2nd	→ X
	51bp	10/11	300 → 30 ng/ul
	51bp	pH 5 10/12	10 ug → 1 ug/ul
	51bp	pH 6 10/12	4 ug → 400 ng/ul

Set-up Samples for westerns -
Use.

①	HTPAN08	185bp	60 ng/ul	= 100 ng
②		pH 5 10/12	1 ug/ul	= 0.1 ul
③		pH 6 10/12	0.4 ug/ul	= 0.25 ul
④	→	Galk	uninduced	Control

Run 3 Gels - Marker 1, 2, 3, 4, Marker 1, 2, 3, 4, Marker 1, 2, 3, 4
will get a total of 9 blots.

0/26/95

DO Different Amounts of Samples

	50ng	200ng	500ng
185bp	0.83	3.33	8.33
pH 5 10/12	0.05	0.2	0.5
pH 6 10/12	0.125	0.5	1.25

for all samples:

500 μ l	H ₂ O
50 μ l	0.15% NaDOC
75 μ l	50% TCA

Mix well

Spin 10 min

Remove Supernatant

Resuspend different Quantities of protein in 10 μ l 0.2N NaOH

Resuspend the 100ng Samples in

45 μ l 0.2N NaOH

Add equal Volume 2X dissociation Buffer

Run 10 μ l of 100ng Samples per lane and all of different Quantities

	185bp			pH 5 10/12			pH 6 10/12			
Marker	50ng	200ng	500ng	50	200	500	50	200	500	marker

Run at 100 V 1.3 hrs.

Transfer Gels to Nitrocellulose

Western transfer - See pg 34

10/5/95

10/26/95

After transfer is complete.
 Mark - Markers
 Out into Strip plots.
 Store at 4°C in Western Block
 Buffer. 3% BSA Fraction IV
 0.02% Na Azide in PBS.

Get Rabbit Ab from Young Sa Kim

Rabbits 11940 & 11941
 Large Bleeds 9/22/95
 10/20/95.

Do Western.

Dilute Ab 1:200 in Western Block
 Buffer.

(1)	11940	9/22/95
(2)	11940	10/20/95
(3)	11941	9/22/95
(4)	11941	10/20/95.

Incubate at RT w/ Shaking
 overnight.

"PAPER Chase"

10/27/95

Western -

Pour off 1° Ab.
 Rinse filters in 1x PBS.
 Wash filters in 1x PBS.
 5 min at RT

10/27/95

Add 20 Ab. in 1x PBS
 1:1000 of Anti Rabbit Peroxidase
 Conjugate

Incubate at RT w/shaking
 for 3 hrs

Pour off 20 Ab

Rinse filters

Wash filters 1x PBS

Wash filters 1x PBS 5min at RT

w/ Shaking

Pour off wash

Wash filters 50mM NaH_2PO_4 pH 7
 at RT 5min w/shaking

Add Substrate:

12.5 ml

50mM NaH_2PO_4 pH 7

2.5 mg

β -NADH

2.5 ml

Phenol

8.5 ml

H_2O_2

3.75 ml

NBT

Mix well

Add to filters

Incubate

till desired color develops

Stop Rxn with di H₂O

1-Marker 2-185bp 3-pH5 4-pH6 5-Galk



11490
9/22



11490
10/20



11491
9/22



11491
10/20

10/27/95

Set up new gels for HTPAN 08.

Follow same Guidelines as in p10
 10300000 - 100ng of protein
 per well.

Store Blots in Western Block Buffer
 at 4°C.

PA2 + PQE 60/70 Vectors.

Spin 15min
 Remove Supernatant
 Wash pellets 800ul 70% Ethanol
 Spin 5min
 Remove Supernatant
 Allow Pellet to Dry at RT for
 30min
 Resuspend pellets in 50ul TE
 Run ligation with 1 Kb ladder



- 1 Bam HI / Bgl II
- 2 Bam HI / Nco I
- 3 Bgl II / Bam HI
- 4 Bgl II / Nco I
- 5 Nco I / Bam HI
- 6 Nco I Bgl II
- 7 Bam HI / Bgl II
- 8 Bam HI / Sph I
- 9 Bgl II / Bam HI
- 10 Bgl II / Sph I
- 11 Sph I / Bam HI
- 12 Sph I / Bgl II

- 13 Asp 718 / Bam HI
- 14 Bgl II / Bam HI
- 15 Kpn I / Bam HI
- 16 Bam HI / Asp 718
- 17 Bam HI / Kpn I
- 18 Bam HI / Bgl II

STYL Vector #3 BOR
 -20C

10/30/95

Dilute 1st Ab to see how far to dilute protein & antibodies

Use Blots from 10/26 & 10/27

Blot	1 →	1:200
	2 →	1:300
	3 →	1:400
	4 →	1:500
	5 →	1:750
	6 →	1:1000

10ml Western Block Buffer

60 μ l
33.3 μ l
25 μ l
20 μ l
13.3 μ l
10 μ l

use 1st Ab from #11490 10/20/95
Incubate O/N at Room temp
with Shaking.

10/31/95



Remove 1st Ab
Wash Blots 1x PBS
Wash Blots 5 min in 1x PBS
at Room temp w/ Shaking
Pour off Wash
Add 2nd Ab

Goat-Anti-Rabbit Peroxidase
1:1000 in PBS
Incubate at Room temp w/ Shaking

Pour off 2nd Ab
Rinse Blots 1x PBS
Wash Blots 5 min in 1x PBS
at Room temp w/ Shaking

10/31/95

Pour off wash
Washed filters 50mM Na_2HPO_4 pH 7
at Room Temp for 5 min

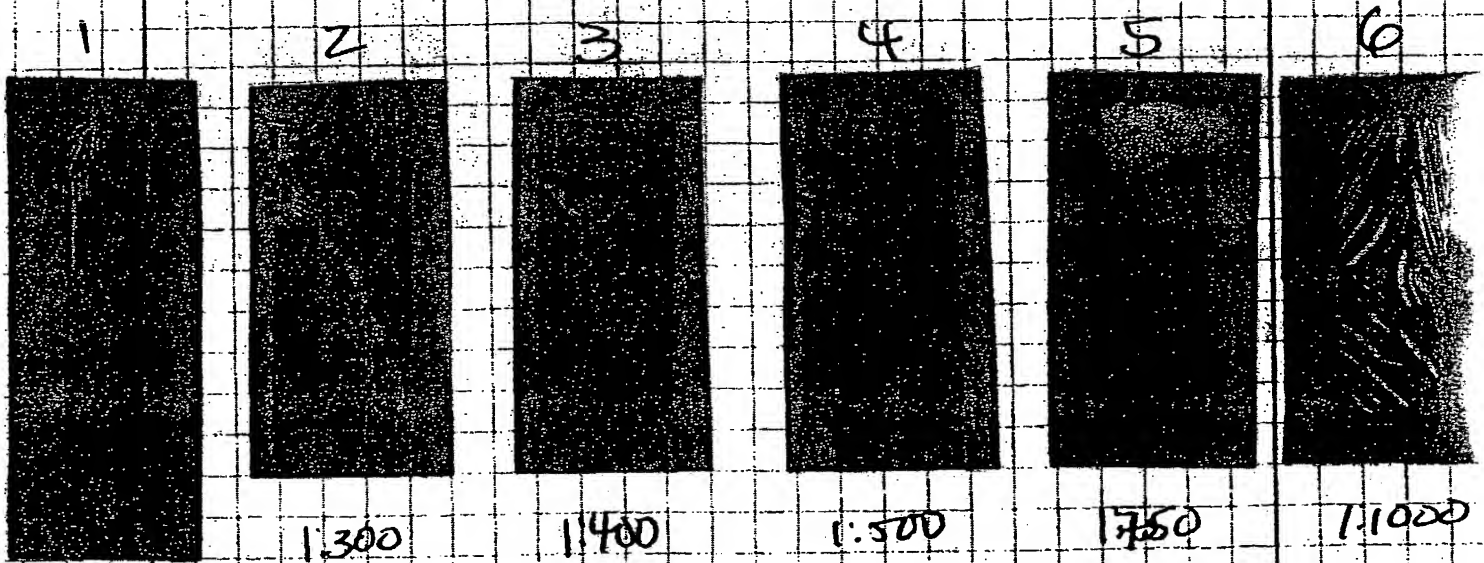
Pour off wash.

Add Substrate:

12.5 ml 50mM Na_2HPO_4 pH 7
25 μl phenol
25 mg β -NADH
8.5 ml H_2O_2
37.5 ml NBT

Add to filters

Incubate to develop color -
Stop with the addition of
 LiH_2O



1:200

looks like even at 1:1000 dilution
protein looks good

11/1/95

Add 1° Ab at 1:1000 to Western
from 10/26/95 (pg 64)

Incubate overnight at room temp
with shaking

Will need to plate libraries to screen
for full length gene of

HSXCB49 - interferon
currently it is Henrik Olsen's
project

11/2/95

Remove 1° Ab

Wash Blots 1x PBS

Wash Blots 5 min 1x PBS at

Room Temp w/ shaking

Pour off Wash

Add 2° Ab at 1:1000 Dilution

in 1x PBS - Anti Rabbit Peroxidase

Incubate at Room Temp w/ shaking

for 3 hrs

Pour off 2° Ab

Rinse filters 1x PBS

Wash filters 1x PBS 5 min at RT

with shaking

Pour off Wash

Rinse filters in 50mM NaH₂PO₄ pH 7.0

Wash filters in 50mM NaH₂PO₄ pH 7.0

for 5 min at Room Temp with

shaking

Pour off Wash

Add Substrate

11/2/95

Substrate

12.5 ml
25 mg
25 μ l
8.5 μ l
37.5 μ l

80 mM NaH_2PO_4 pH 7.0
 β -NADH
phenol
 H_2O_2 (30% solution)
NBT

Add Substrate to filters
Allow Color to develop to desired
Darkness.

Stop Reaction with d H_2O
Allow Filter to air Dry on Whatman
paper



lanes:

- 1 - Marker
- 2 - 50 ng } HTPAN08
- 3 - 200 ng } 185bp ATG
- 4 - 500 ng }
- 5 - 50 ng } HTPAN08
- 6 - 200 ng } 51bp ATG
- 7 - 500 ng } pH 5 10/12
- 8 - 50 ng } HTPAN08
- 9 - 200 ng } 51bp ATG
- 10 - 500 ng } pH 6 10/12
- 11 - Marker
- 12 - Blank Uninduced

Titer HBT library \rightarrow

1:10 \rightarrow 1:100 \rightarrow 1:1000 1:10⁴ 1:10⁵ 1:10⁶ 1:10⁷

plate out ~~1:10~~ 1:10⁶, 1:10⁵, 1:10⁴

11/2/95

Incubate 200ml LB + 10mM MgSO₄, 2mM
Maltose with Frozen Stock of
LE392
Incubate 37°C w/aeration Overnight

11/3/95

plate out HBJ, H5X, HFL
20 plates - 30,000 plaques/plate

HBJ - Titer: 60,000 $\Rightarrow 6 \times 10^5$ /ml
H5X - 2.4×10^9 /ml
HFL - 2.2×10^9 /ml

Spin LE392 ON Culture

3K, 15 min

pour off Supernatant

Resuspend Cells in 10mM MgSO₄
OD600 = 1.0

TO 1ml Cells add phage

HBJ - 1ul

H5X = 0.00025 ml

HFL = 0.00027 ml

Incubate Cells & phage 37°C 15 min

Heat LB + 0.75% Agarose in Microwave to
melt all of Agarose.

Aliquot 7ml per tube and place in
30°C Hot Bath to Cool

After Incubation of Cells & Phage is Complete

11/10/95

Computer Work

11/10/95

11/13/95

Develop HSX.CB49 1° Screens
again.Looks like there are a couple of ⊕
picks into 500ul 5m BufferHSX - 1, 2, 3, 4
HFL - 1
HBJ - 1PCR to test if insert is there
Get FP03 primer from Laurie (LAI)

HSX.CB49.FP03 (3.2µm)	2	
M13R (3.8µm)	0.02	⊕ Control
10x dNTP	5	plasmid
10x PCR	5	DNA
H ₂ O	35.8	
Tag	0.2	⊖ Control
Phage	2	H ₂ O
	60ul	

PCR - PROGRAM 58.

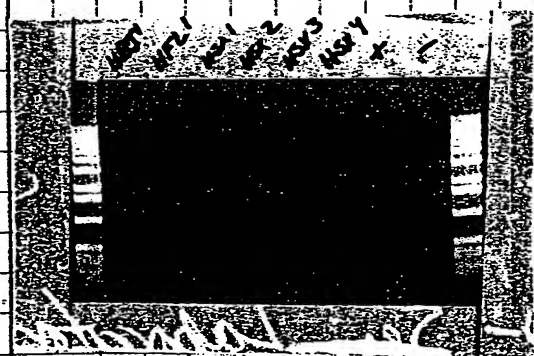
95°C	5min	
95°C	1min	} 30x
55°C	1min	
72°C	2min	
72°C	7 1/2 min	
4°C	Hold	

11/13/95

Plate 2° -
300ul LE392 OD₆₀₀ = 1
+ 2nd of 2° phase

Incubate 37°C 15 min
Plate onto 150 mm NZY plates
in 7 ml NZY + 0.7% agarose.
Incubate 30°C ①/N

Run 1% TAE gel with 1 kb ladder



No thing worked
even \oplus control
Get another
primer from
Laura & try
again.

Revised Primers -

Some -
Diluted to 3.2 pmol/l for
Sequencing

Juan N. → Requested Rabbit Antibody
Sera -
I gave him 1 ml each.

11491 - 10/22/95

11490 - 10/22/95

11/17/95

Pick 2° (+) clones of HSK-

~~10/2/95~~

Only HSK 2° - 3 had any (+).

Picked 12 into 200 µl SM Buffer

Do PCR.

PCR Prog 58.

M13R	0.05
M13F	0.05
10x	10
10x	10
H ₂ O	77.6
Taq	0.3
Phage.	2
	100

95°C	5min	} 30x
95°C	1min	
55°C	1min	
72°C	2min	
72°C	7 1/2 min	
4°C	Hold	

Run 10 µl on gel



Alan Miller
11/29/95

11/20/95

Analyzed sequence of

egf clones - They all blast as
part of the egf/Agf family.

Worked on HOUCAIT clone - see #289

90

11/22 - 11/26 Out of office

11/27/95

Carrie Received clones from
finishers

DATE 11-21-95

CLONE PROCESSING SHEET

Clone I.D. HOAA046, HELDQ06, HCEEX61, HOEDF83, HTEBY23
Requested By Steve Ruben
Transfer Folder 11-17-95
Processed By AMG

(transfer folder 11-20-95)

DATE 11-20-95

CLONE PROCESSING SHEET

Clone I.D. ATEGS 33
Requested By STEVE RUBEN
Transfer Folder 11/20/95 TRANSFER
Processed By JTM BCI

Inoculate 250 ml TBFAmp with
cultures

Carrie Sick so inoculate HEPCU2
for her.

Inoculate B7C O/N w/ aeralant

12/4/95

HNEDJ57

95-117	0.08	4.96
M13R	0.02	1.24
10x dNTP	3.2	198.4
10x PCR	3.2	198.4
H ₂ O	22.3	1382.6
Taq	0.3	18.6
@ Prage	2	
	32	

62x

pl645
should
Be HSBV83!
should be OK
Give CS117a
up for HSBV83
and 117b
for HNEDJ5

Rec'd Primer for HTPAN08504 516p + PAGE

3' Hind III #14388	2	24
5' Nco I #2888	4	48
10x dNTP	3.2	38.4
10x PCR	3.2	38.4
H ₂ O	16.3	195.6
Taq	0.3	3.6
DNA (conjug) 4	1	10
	32	7 32ul / Tube

Run PCR Prog 58

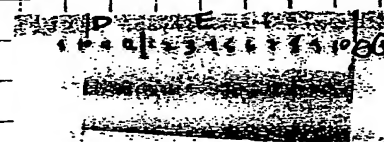
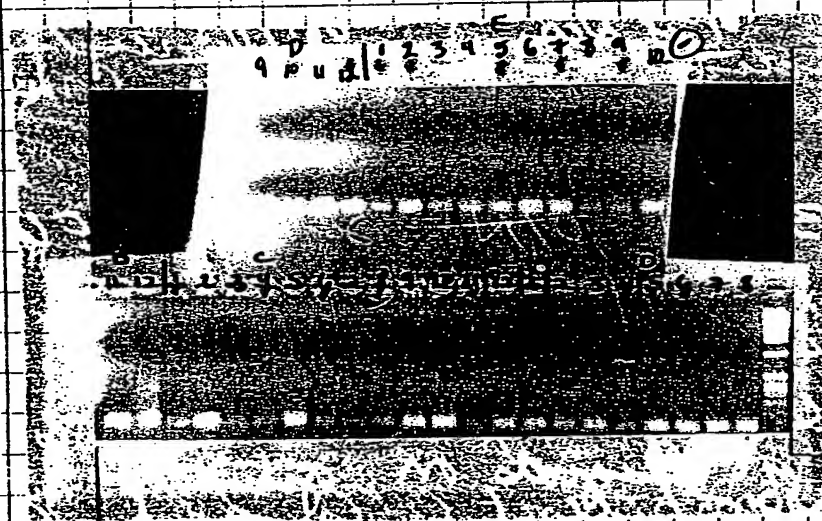
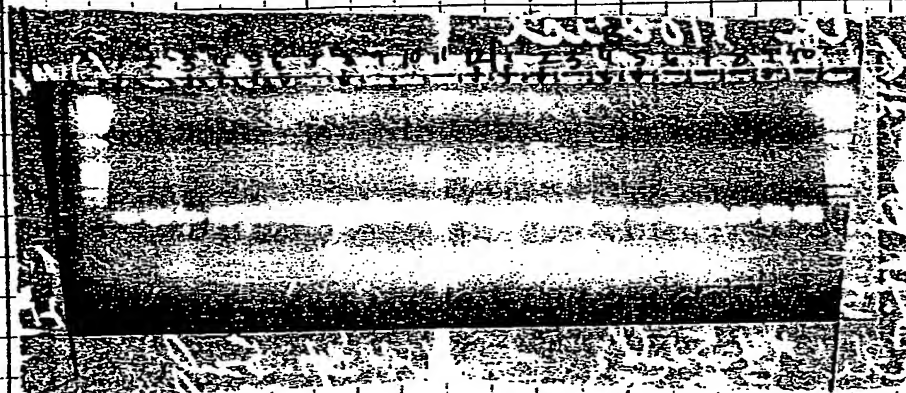
95°C	5 Min
95°C	1 min
55°C	1 min
72°C	1 min
72°C	7 1/2 min
10°C	Hold

Run 10ul orig w/ 1kb ladder

Run 2ul of HTPAN08 PCR

1

12/4/25



Pick + for 2° Screening

A-1	A8	B5	C8	D12	E9
A-3	A12	B9	C11	E1	
A-4	B1	C1	D4	E2	
A5	B2	C4	D5	E5	
A-6	B4	C6	D6	E7	

Dilute 1:100 plate 10.1 into 100mm
 NZ4 Plates
 Fluorobate 30°2 O/N

12/4/95

PEG PD PCR Product

ethanol wash

Resuspend pellet in 80ul TE

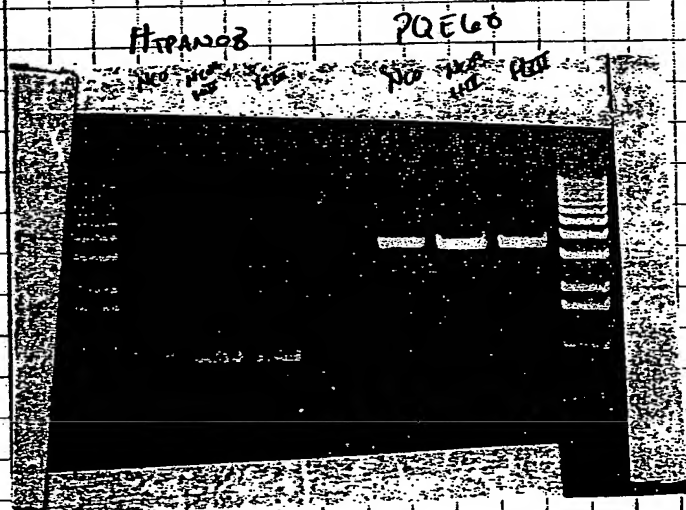
Set up Digestion

	NCO	Nco/HMT	HMT
10X#4	#4-5ul	44-5ul	#2-5
H ₂ O	29ul	29ul	29
DNA	15ul 100	15ul	15
Enzyme	1ul Nco	0.5/0.5	1.0 HMT
	50ul	50ul	50ul

Incubate 37°C O/N - Set up PDE60
 Digests the same as above

12/5/95

Run 10ul of Digests on gel with
 1kb ladder



12/5/95

Incubate ligations at 16°C Overnight.

HNEJ57 2° did not grow dense enough
replate
at 1:500 plate 10ul.
Incubate 37°C 5 hrs.

Most plates look good.
a couple look like they need to
be replated at a higher concentration
Incubate 30°C O/N.

12/6/95

Transform ligations into Chemically
competent M15 up 4 cells

Thaw Chemically Competent M15 Cells
on ice

To 100ul Competent Cells add 10ul
of ligation reaction

let sit on ice 1 hour

Heat 42°C for 1 min

Chill on ice

Add 400ul LB

Incubate 37°C 1 hr

plate 150ul onto LB + Amp plate.

Incubate 37°C O/N

④ Control pQE60 plasmid DNA

HNEJ57 ~~HA~~ should be HSA BV83

2° grew OK.

Chill plates at 4°C 1 hr

12/8/95

Do plate lifts w/ orientation marks.

Denature lifts 2 min
0.5 M NaOH / 1.5 M NaCl

Den Neutralize 5 min
0.5 M Tris pH 8 / 1.5 M NaCl

Allow filters to Dry at RT.

Prehybridize in 2x PIPES / 50% formamide
overnight at 42°C.

12/7/95

Probe Human Tissue Northern

HE2PM21 → H. Fetal (F) + H. MTN II (H)
HTPAN08 → H. MTN I (H) + Cancer (C)

Preprobe in Express lift 42°C.
2 hrs.

Make Probe -

HE2PM21 Xho / R
HTPAN08 Sma Xho / R
H3HBV83 Xho / R

DNA	3
H ₂ O	21
5X Primer	10
	<hr/> 34

Heat 100°C 5 min
Quick Spin
Place on ice

12/7/95

Add
 5X P^{32} dATP Buffer 10
 3P dATP 5
 Rlenom 1
 5ul

Incubate 37°C 8 min
 Spin through A-25 Column
 Count 1ul

	SAM	POS	CH	CPM	
HE2PM21	1	135	1	787036.00	70ul
HSABV83	2	136	1	693160.00	70ul
HTRAND8	3	137	1	674388.00	70ul

Add 50ul Salmon Sperm DNA

to probe
 Heat 100°C 5 min
 Quick Chill

Quick Spin
 Add probe to HE2PM21 Northern S
 and HTRAND8 Northern S

Incubate 42°C 4 hrs

Wash Northern S
 3X 65°C 0.2X SSC / 0.1% SDS

Put on film
 -80°C overnight

For HSHBV83-

Prep of pre-hyb from Filters

Add probe

Add denatured Δ probe to hyb

Incubate 42°C O/N

12/7/95

HIPA08 + PDE 6

Pick colonies into LB + Amp Kan
media in 96 well dish

Pick 12 each of 1-9
6 each of 10-15

Incubate 37°C w/ aeration 3 hrs
Set up PCR

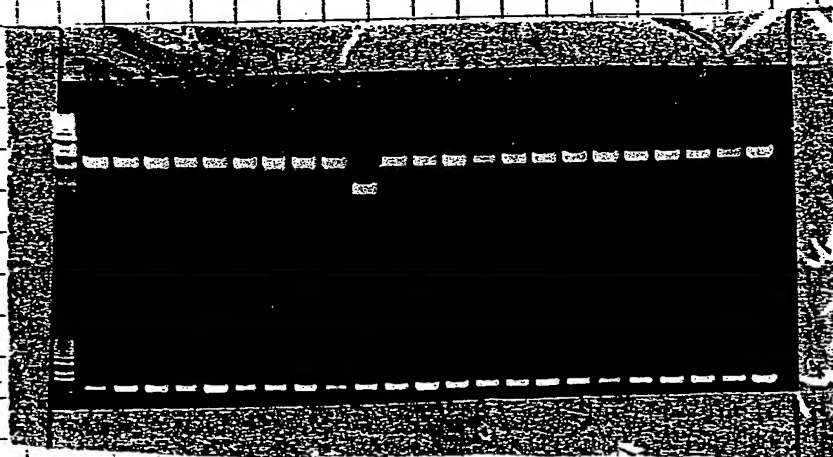
		x 150x
5' PDE	0.2	30
3' PDE	0.1	15
10x PCR	3.2	480
10x dNTP	3.2	480
H ₂ O	28.1	3465
Temp	0.2	30
Cult.	2	
	32	30ul / well.

PCR Prog 66.

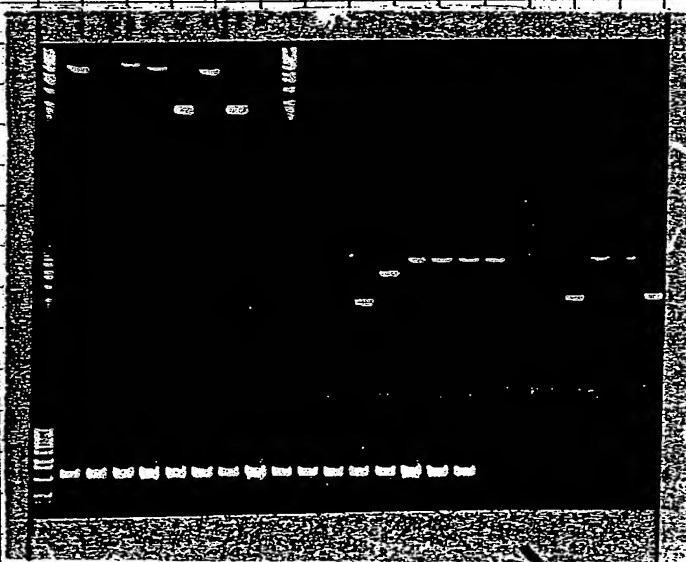
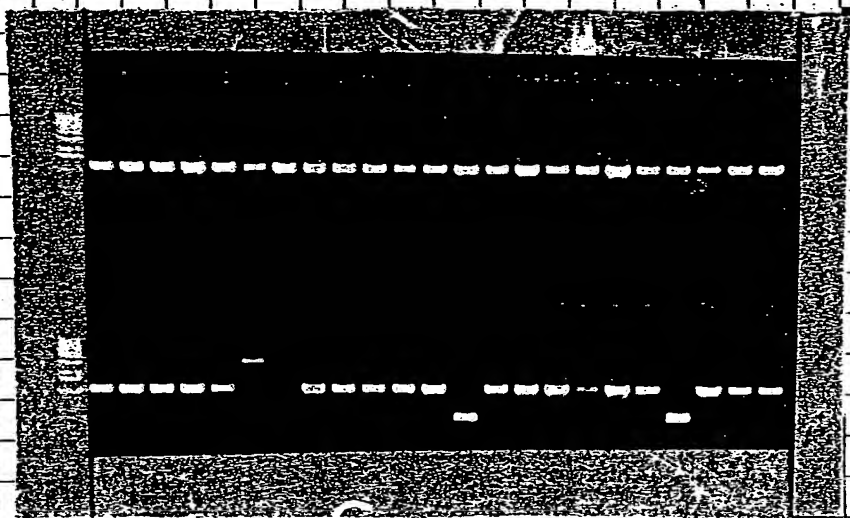
95°C	5min	} 30x
95°C	20sec	
55°C	20sec	
72°C	1min	
72°C	7 1/2 min	
4°C	Hold	

⊕ Control
PDEd
⊖ Control NO DNA

Run 10 ul on gel w/ 1 kb ladder



12/7/95



Pick Clones for
Boiling Miniprep

-1	3-8	5-4	7-1
-2	3-10	5-7	7-2
-3	3-11	5-6	7-3
-4	3-12	5-9	7-4
2-5	4-1	6-9	8-5
2-6	4-2	6-10	8-6
2-7	4-3	6-11	8-7
2-8	4-4	6-12	8-8
9-9	9-11	9-10	9-12

into 5ml TB+amp/Ka

12/8/95

Boiling Mini prep.
Spin 2nd Culture 5 min
Remove Supernatant

12/8/95

Resuspend pellet in 800 μ l STE

RNase & lysozyme.

Heat 100°C 5 min

Spin 15 min

Transfer 750 μ l Supernatant to
fresh TubesAdd 750 μ l PEG / NaCl

Mix well

Spin 15 min

Remove Supernatant

Wash pellet 1000 μ l 70% Ethanol

Spin 5 min

Remove Supernatant.

Allow pellet to air dry.

Let go too long - 2 hrs

Pellet very hard to resuspend.

Add 200 μ l TE to pelletStore 4°C over weekend.

ASHBV83

Wash filters 0.2XSSC / 0.1% SDS

3X 65°C

Put filters on film.

-8

Northers.

Developed Film.

Very faint

Reexpose -80°C over the weekend.

4/11/95

HSHB183 2°

Pick 2° plugs into 200 µl 5m Buffer.

Screen PCR.

95-112	0.1
M13R	0.05
10x dNTP	5
10x PCR	5
H ₂ O	34.6
Taq	0.25
Phage	5
	<hr/> 50.0

95-114	0.1
M13R	0.05
10x dNTP	5
10x PCR	5
H ₂ O	34.6
Taq	0.25
Phage	5
	<hr/> 50.0

PCR Program 58

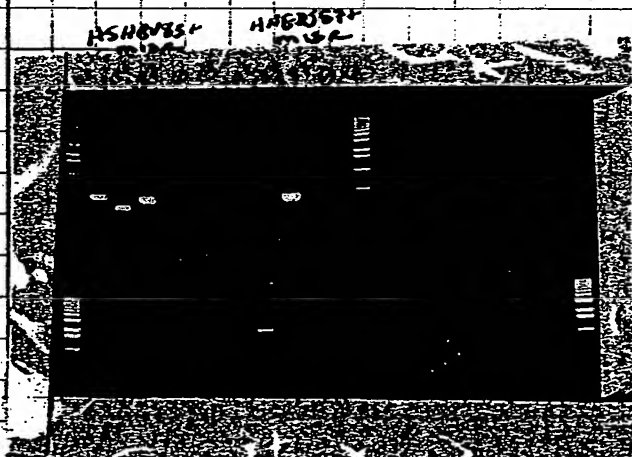
95°C	5 min	} 30X
95°C	1 min	
55°C	1 min	
72°C	1 min	
72°C	7 1/2 min	
4°C	Hold	

Check Primer pairs on HSHB183 Plasmid DNA HNEJ57 Plasmid DNA

Use 91-87 which should be HNEJ57 Specific

Run 10 µl on gel with 1 kb ladder

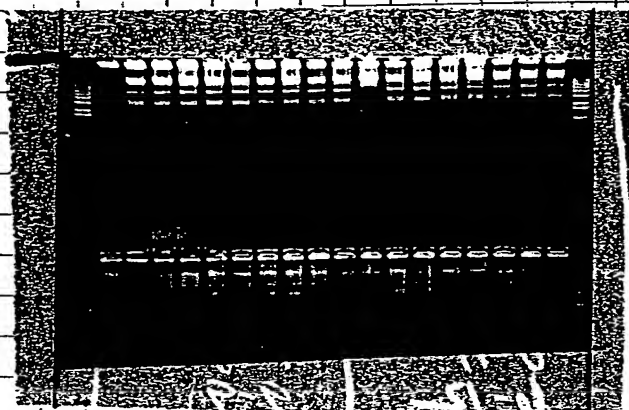
134
13-2
13-3
15-1
15-2
15-3
17-1
20-1
20-2
20-3



Insulate
XL-1 MRF
SOLR
LE392
to Excise

Resuspend HTPANOR + PQE6 clone
in 200ul TE
Run 2ul on gel w/ 1kb ladder

12/11/95



Setup Digests

DNA	5	38.5
10X#4	3	1.14
H ₂ O	218	8.284
N ₄ O	0.1	3.8
1+III	0.1	3.8

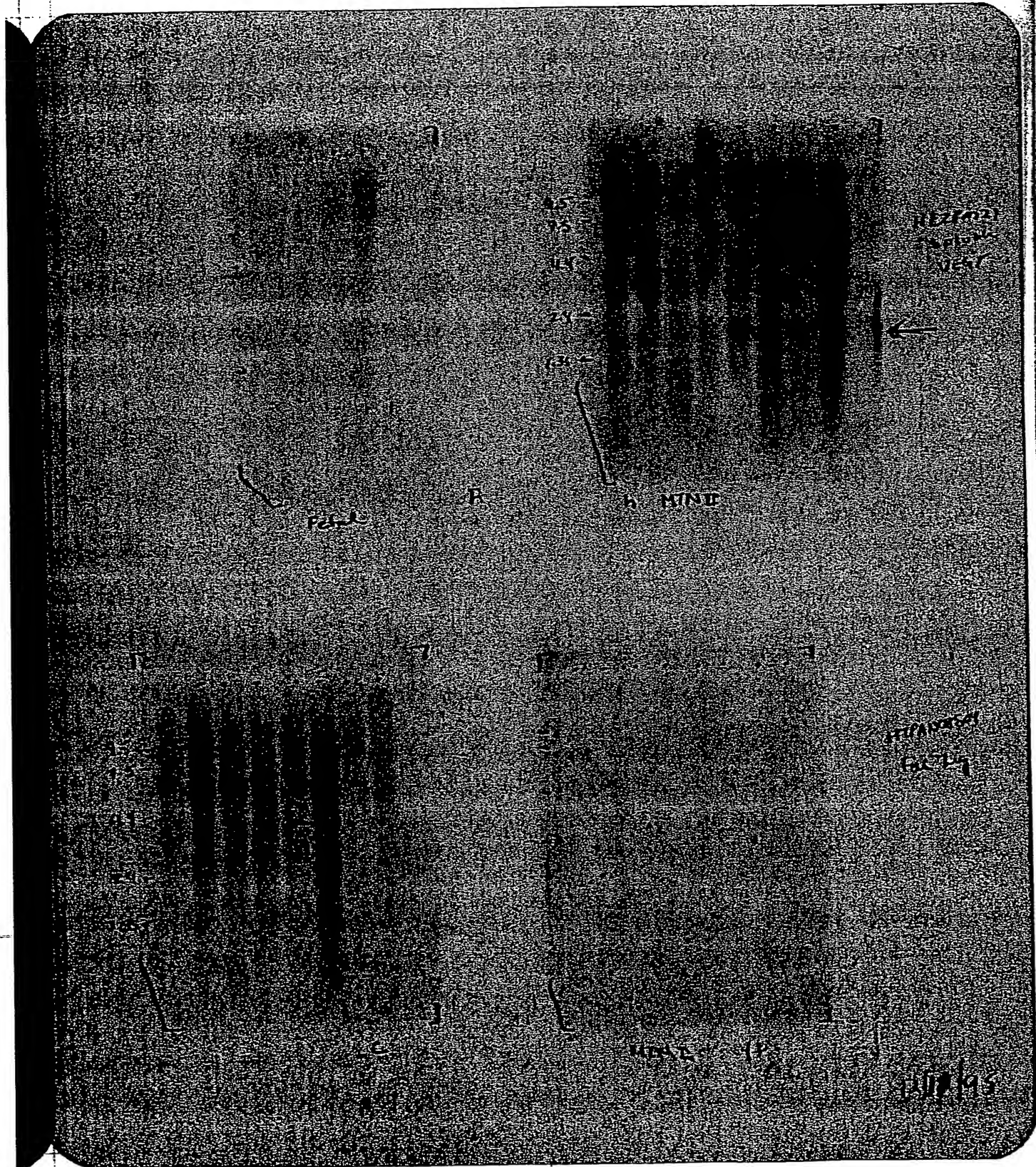
Incubate ³⁰
at Room Temp
overnight

Small Scale induction:
Incubate 500ul of LB + Amp/Kan with
50ul of O/N culture of HTPANOR + PQE6.

Incubate 37°C 1 1/2 hrs
Add 11ul of IPTG (100mM) to 2mL.
Incubate 4 hrs w/ aeration 37°C.
Spin culture 3m.
Resuspend pellet 40ul H₂O.
Add 40ul 2X dissociation Buffer
Store -20°C or Run on 1% Gel.

Develop Northern.

Difficult to say if there is anything
Strip blots 0.5% SDS in 250ul H₂O
Heat 5min in microwave.
Place blots in 0.5% SDS.
Incubate 10 min.
Repeat until NO counts can be
heard



REZKAZ
TAKHLEH
WEST



MINI

REZKAZ
TAKHLEH

5

8.4
8
8

60

10/95

5

1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

Resuspend HTPANOR + PQEC clon

12/11/95

1

8.4

8

8

0

12/12/95

Run 10 μ l of induced Proteins on
15% SDS-PAGE Gel.

	Gel 1	Gel 2	Gel 3	Gel 4
Ln 1	Marker	3-10	5-9	8-6
2	1-1	marker	6-8	8-7
3	1-2	3-11	Marker	8-8
4	1-3	3-12	6-9	Marker
5	1-4	4-1	6-10	8-9
6	1-5	4-2	6-11	9-8
7	2-5	4-3	6-12	9-9
8	2-6	4-4	7-1	9-10
9	2-7	4-5	7-2	9-11
10	2-8	5-5	7-3	9-12
11	2-9	5-6	7-4	9-9 UN
12	3-8	5-7	7-5	2-5 UN
13	3-9	5-8	8-5	4-3 UN
14	2-5 UN	4-3 UN	6-12 UN	6-12 UN

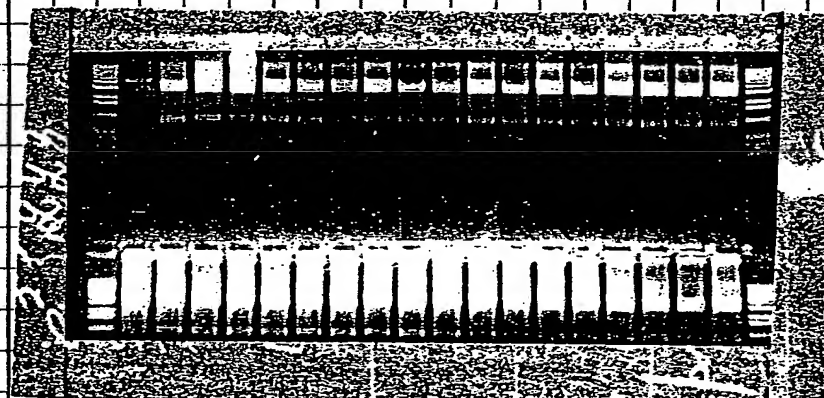
Run 150V 1 1/2 hrs.
Stain 30 min at 57C.
De-STAIN 7hr at 37C
Dry Gels.

12/12/95

Can't tell Do Small Scale
inductions

HTPANOS + PDE6

Nco / HAT digests



All clones
look correct
submit
for Sequencing

Rescul HSHB183 20-1

20ul phage + 100ul XL-1 Blue MRF $OD_{600} = 1.0$

+ 10ul Ex Assist

Incubate $37^{\circ}C$ 15min

Add 2ml LB

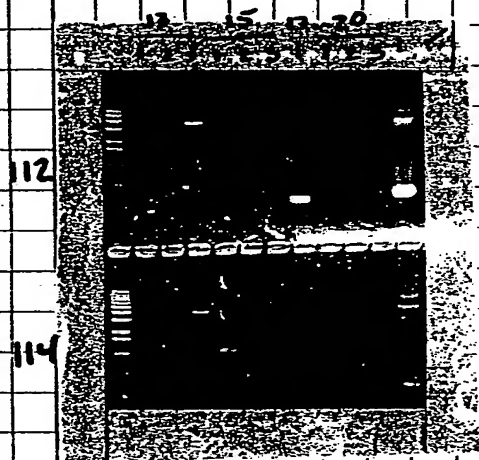
Incubate $37^{\circ}C$ 2hrs

Heat $68^{\circ}C$ 15min

Spin - Plate 1ul / 15ul / 10ul / 20ul 50ul

116

12/15/95

Run 10 μ l on gel w/ 1K ladder

On Monday, Plate

13-3

15-1

20-2

17-1

HSHB183

HTRANO3 + PGE6.

PCR 1-1, 2-5, 3-8, 4-1, 5-6
 w/ 1 μ l T7 PGE60/70 and 3' HLT
 8 tubes of 32 μ l

T7 PGE 60/70 (10 pmol/ μ l)

HTRANO3 3' HLT

10 \times dNTP10 \times PCRH₂O

Taq

DNA (5 ng/ μ l)

1

2

3.2

3.2

20.3

0.3

2

3.2

40 \times .

40

80

128

128

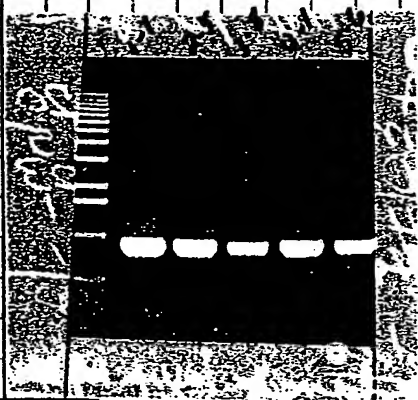
812

12

PCR Program 66.

Run 5ul on gel w/ 1kb ladder

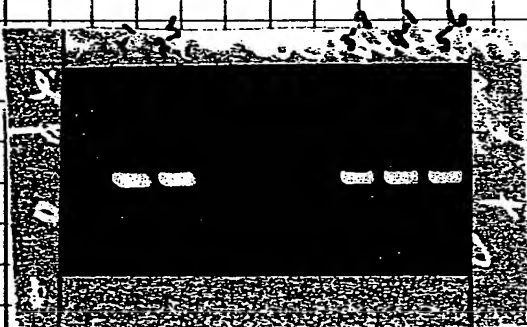
12/15/95



Combine the 8 tubes
Ppt Reaction with
Equal Volume
PEG/Wash

Wash pellet 100ul
70% Ethanol
Allow Pellet to Air
Dry
Resuspend pellet in
60ul TE

Run 1ul on gel with 1kb ladder



Submit 1-1 + 2-5
for TNT - T7

along with HESCT26
for TNT - T3

12/18/95

HSABV83-

Excision + Rescue

200 ul SAR OD₂₆₀ = 1.0
10 ul Rescue Stock

Incubate 37°C 15 min

Develop

12/21/95

Jit

HSKBNO

HMSA

3.10

HMSA

PQE

GP

10X

T4

Clonk

H

Incubate 4°C Ours Break

12/22/95 1 Personal Day OFF

12/25/95 Christmas Day OFF

12/26 - 12/29/95 Company Break

1/1/96 New Years Day OFF

1/2/96 1 Vac Day OFF

1/3/96 - 1/5/96 3 Personal Days OFF

1/8/96 - 1/10/96 Company Closed Due to weather

1/11/95

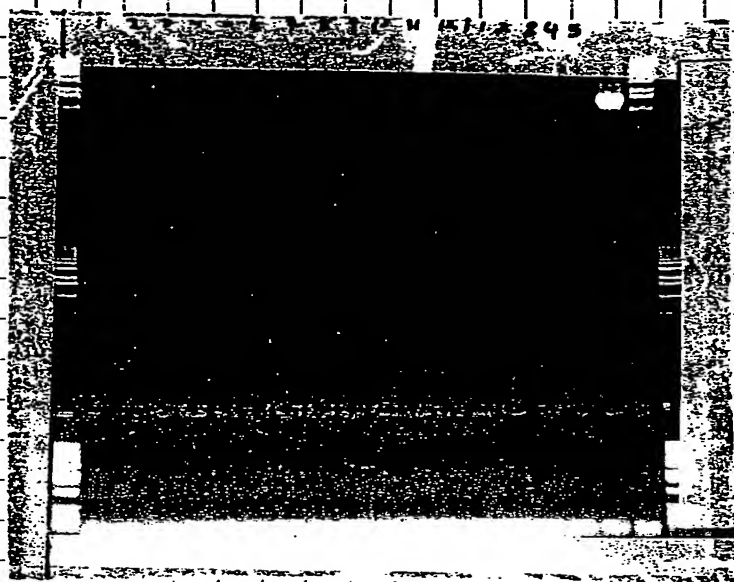
Write in note book Write progress report for Steve

10/20/95

Run 10x10 on gel with

10 Kb ladder

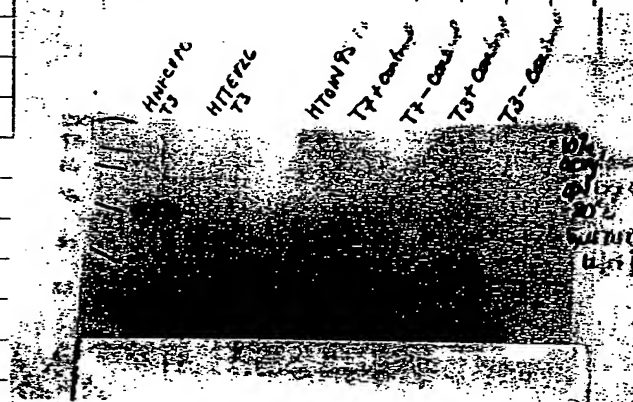
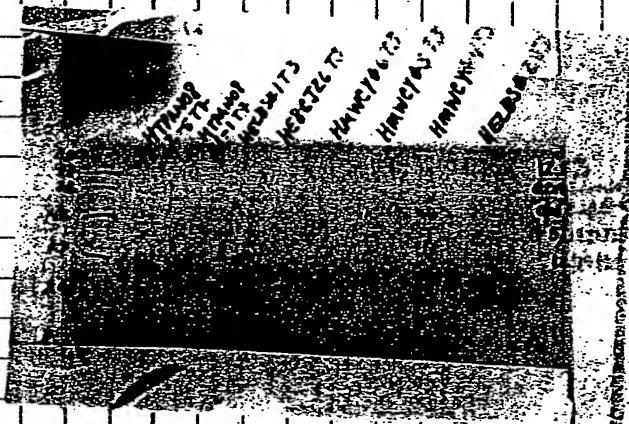
H VSAF 22 + PAR



TNT RESULTS 12/19/95			
INVESTIGATOR	SAMPLE NAME	EXPECTED PRODUCT SIZE KDa	OBSERVED PRODUCT SIZE KDa
ANN KIM	HTPAN08+PQE6 #1-1	33	33
ANN KIM	HTPAN08+PQE6 #2-5	33	33
ANN KIM	HE8CJ26	30	NO PRODUCT OBSERVED (background band at 30)
JING SHAN HU	HMWCY #6	30	NO PRODUCT OBSERVED (background band at 30)
JING SHAN HU	HMWCY #5	26	NO PRODUCT OBSERVED
JING SHAN HU	HMWCY #4	33	NO PRODUCT OBSERVED (background band at 30)
JING SHAN HU	HELBS #2	37	MAJOR BAND AT ~25 (others at 29 & 36)
JING SHAN HU	HELBS #1	30	MAJOR BAND AT ~25 (others at 29 & 36)
BRENT KREIDER	HNFCV70	92	TRIPLET BANDS AT 44, 46 & 48, MINOR BAND AT 30
BRENT KREIDER	HTTEV26	110	MAJOR BAND AT 36 (others at 30, 40 & 27)
LAURIE INSCORE	HTOIN95	48	NO PRODUCT OBSERVED
T7 POSITIVE CONTROL	DNASE 02-105	33	33 (gel loading problem, but band is visible)
T7 NEGATIVE CONTROL	NO TEMPLATE	NONE	NONE
T3 POSITIVE CONTROL	HCACI93	33	33
T3 NEGATIVE CONTROL	NO TEMPLATE	NONE	NONE

12/20/95 \$

TNT Results Continued



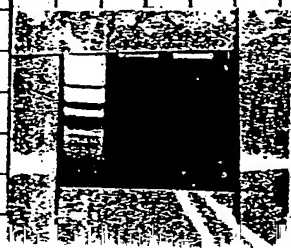
12/20/2011

PPT Drysts of

#MSAF22 3' Delta A₃₀₀ / 3' P_{21.1}
HASKB009 H₂A / SpH₁
w/w equal volume PEG / NaCl
-20 C overnight

12/21/95.

Run ~~the~~ GP 12 Digests on & bone cleaned
fragments on gel.
Looks good.



Develop film - MNT

12/21/95

Set up ligation:

	1	2	3	4	5	6	7	8	9
HSKBN09. HII / Sph I	3	—	—	3	—	—	—	—	—
HMSAF22 3' Delta Asp I Bgl II	—	3	—	—	3	—	—	—	—
HMSAF22 3' ASP / Bgl II	—	—	3	—	—	3	—	—	—
PGE 7 HII / Sph	2	—	—	—	—	—	2	—	—
GP PAZ ASP / Bgl II	—	2	2	—	—	—	—	2	2
10X T4 Buffer	2	2	2	2	2	2	2	2	2
T4 Ligase	1	1	1	1	1	1	1	1	1
H ₂ O	12	12	12	14	14	14	15	15	17

Incubate 4°C Over Break

12/22/95 1 Personal Day OFF

12/25/95 Christmas Day OFF

12/26 - 12/29/95 Company Break.

~~12/31/95~~ 1/1/96 New Years Day OFF

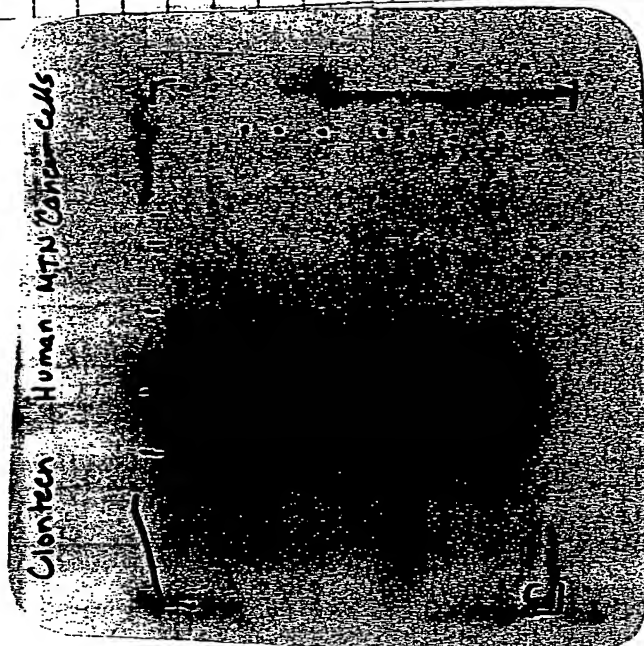
1/2/96 1 Vac Day OFF

1/3/96 - 1/5/96 3 Personal Days OFF

1/8/96 - 1/10/96 Company Closed Due to weather.

1/11/95

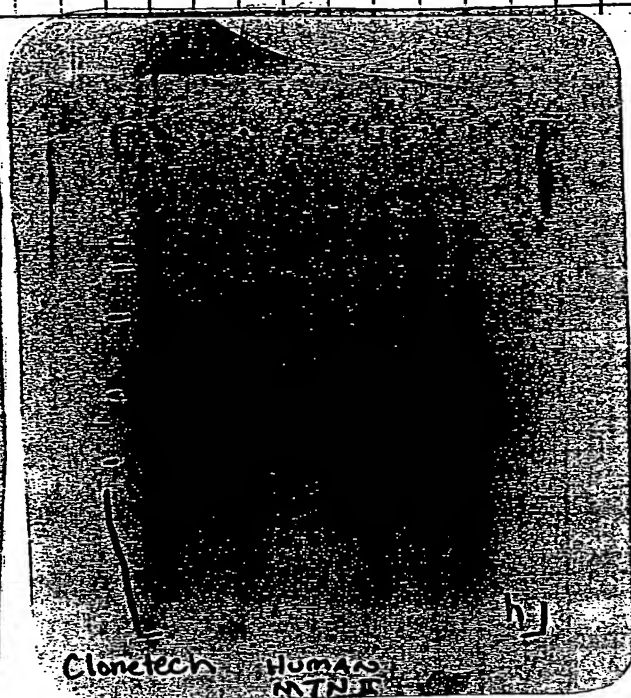
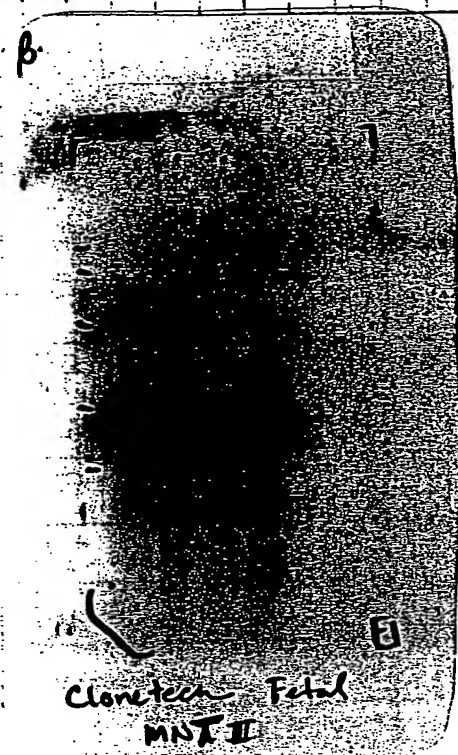
Write in note book - Write progress report for Steve



1/3/96 - 1/5/96 3 Personal Days OFF
 1/8/96 - 1/10/96 Company Closed Due
 to weather.

1/11/95

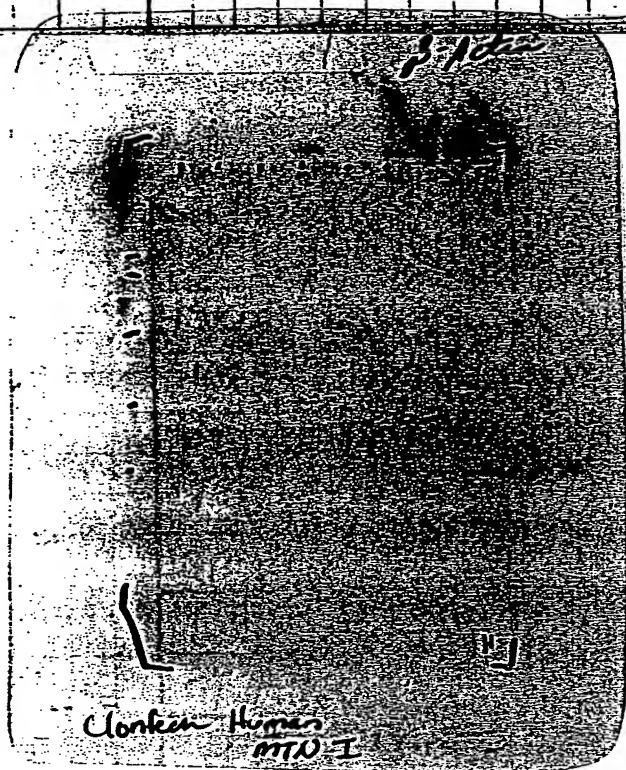
Wrote in note book - Write progress
 report for Steve



1/3/96 - 1/5/96 3 Personal Days OFF
1/8/96 - 1/10/96 Company Closed Due
to weather

1/11/95

Write in note book - Write progress
report for Steve



1/2/96 - 1/5/96 3 Personal Days OFF
 1/8/96 - 1/10/96 Company Closed Due
 to weather

1/11/95

Wrote in note book - Write progress
 report for Steve

1/12/98 Company Closed due
to weather

~~12/15/98~~

1/15/98

Transform ligations

for all PDE7 ligations transform
into Chemically Competent
M15rep4 cells and plate
onto LB+Amp Kan plates

For all BPPA2 ligations Transform
into Chemically Competent
DH5 α - (GIBCO BRL) cells and
plate onto LB+Amp Plates.

Incubate 10 μ l of ligation reaction
with 100 μ l of Chemically
Competent cells

incubate on ice 1 hr

heat 42°C 1 min

Place on ice

Add 300 μ l LB

Incubate ~~on ice~~ at 37°C for 1 hr

Plate onto appropriate plates

Incubate 37°C overnight

1/25/96

Set up T₁T₂ Reactions T₃.

1 H H F F D 13

2 H E T A Q 37

3 H E 2 O T 4 2 S 14

4 H T P B 4 N S 15 (+) control

5 (-) control H₂O only

	1	2	3	4	5
Rabbit Ret. Lip	12.5	12.5	12.5	12.5	12.5
T ₃ Buffer	1	1	1	1	1
AA Omet	0.5	0.5	0.5	0.5	0.5
35S Met	2	2	2	2	2
RNasein	0.5	0.5	0.5	0.5	0.5
T ₃ Polymerase	0.5	0.5	0.5	0.5	0.5
DNA	0.5	0.5	4	4	0
H ₂ O	7.5	7.5	4	4	8
	25	25	25	25	25

Set-up reactions in ice

Incubate T₃ & T₇ Reactions
at 30°C 2 hrs.

Spin 1 min

To 5 μ l 2X Dissociation Buffer add11 5 μ l Rxn

Heat 70°C 5 min.

Quick Spin

Run on gel at 150V 1 1/2 hrs

Stop run. Before Dye front Runs
off gel.

Fix for 30 min at 37°C with

Fixative: 10% OHAc
30% MethanolDry on BioRad Gel Drier between
Cellophane.

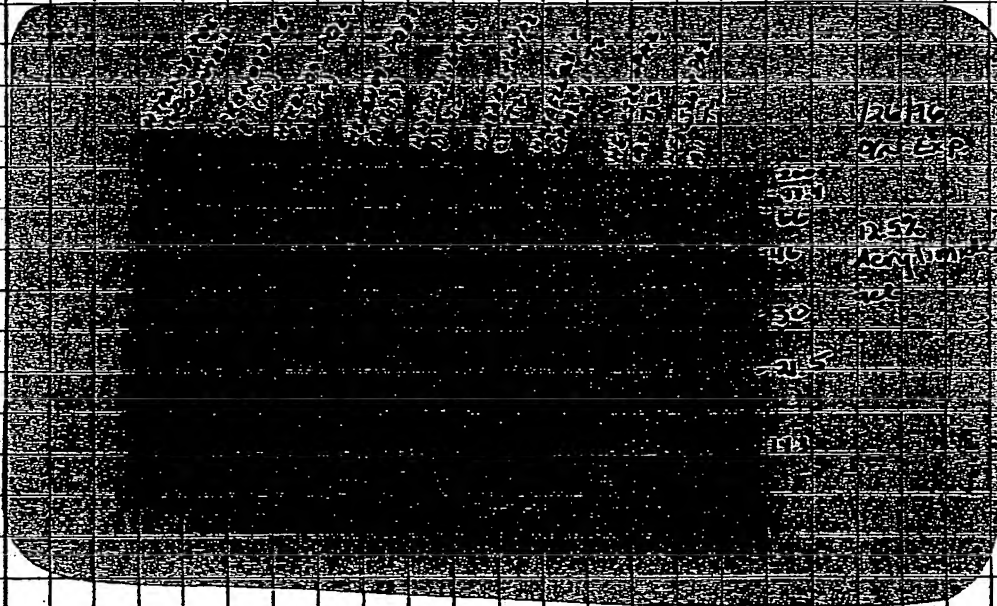
1/25/96

Meeting w/ Henrik Olsen,
 Laurie Unscar,
 Mario Cepeda,
 Camille Fischer,
 Steve Ruben.

Discuss Cloning Core facility
 SOP etc.
 Submitted: Cloning Request
 form for approval of Group.
 Decided on Vectors to have on stock.

1/26/96

Develop TINT



Case

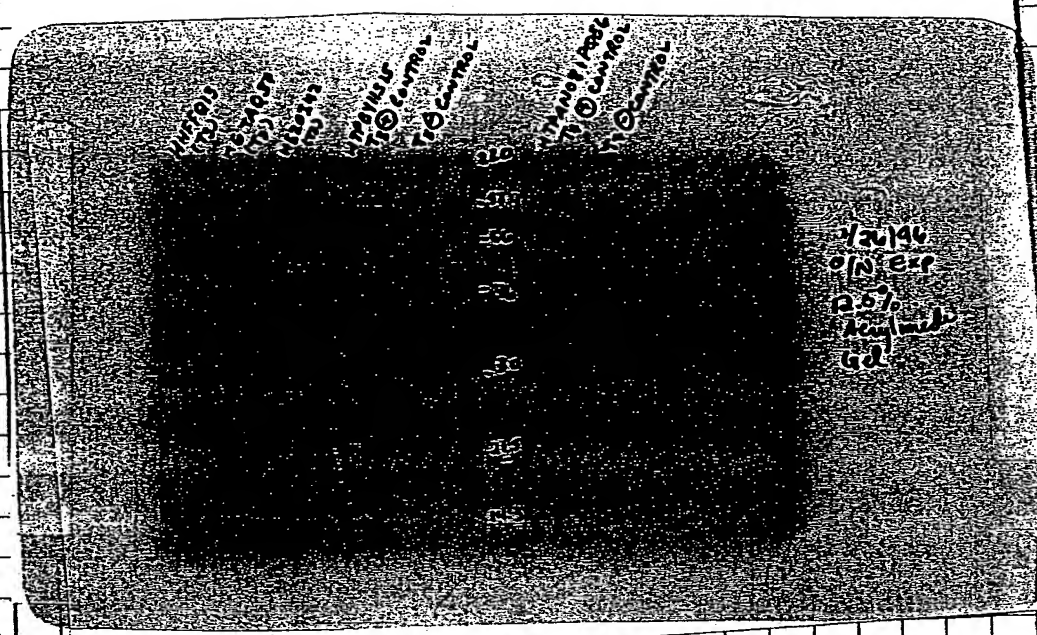
Print

		TNT RESULTS 1/29/96			
INVESTIGATOR	SAMPLE NAME	EXPECTED PRODUCT SIZE KDa	OBSERVED PRODUCT SIZE KDa		
YAJUN CHEN	HETAQ37	51	30 (51 - background?)		
YAJUN CHEN	HHFFQ13	110	NO PRODUCT OBSERVED		
ANN KIM	HSKBN09A1	37	26, 33, 37		
ANN KIM	HSKBN09A5	37	26, 37		
ANN KIM	HSKBN09B1	37	NO PRODUCT OBSERVED		
ANN KIM	HE8CJ26A1	33	33		
ANN KIM	HE8CJ26A5	33	37		
ANN KIM	HMSAF22C1	37	33		
ANN KIM	HMSAF22D1	37	28, 33, 37		
ANN KIM	HMSAF22DELTA3/ASP A2	33	37		
T7 POSITIVE CONTROL	HTPAN08PQE8	33	33		
T7 NEGATIVE CONTROL	NO DNA	NONE	NONE		
T3 POSITIVE CONTROL	HTPBY11S15	94	96		
T3 NEGATIVE CONTROL	NO DNA	NONE	NONE		
	REACTIONS PERFORMED BY:	ANN KIM			

1/26/96

1/29/96

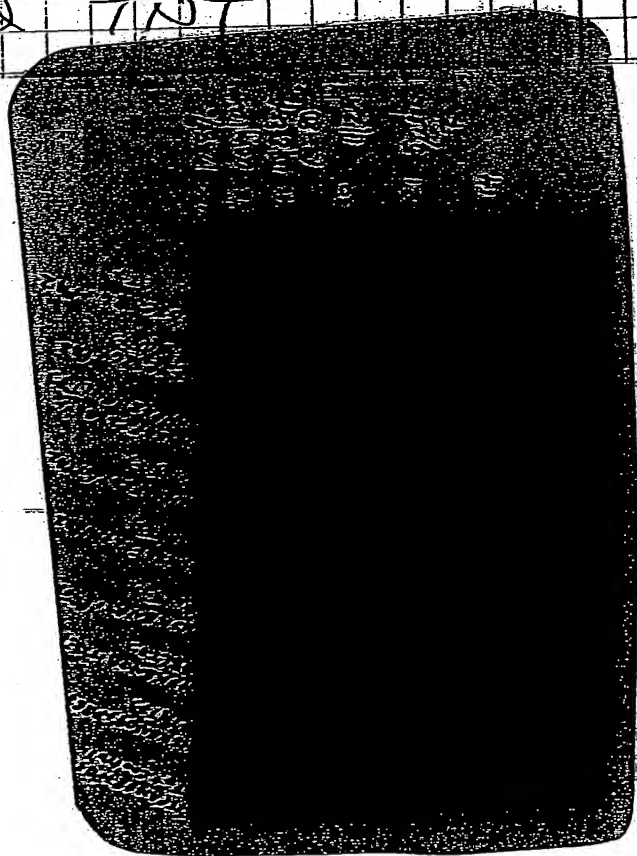
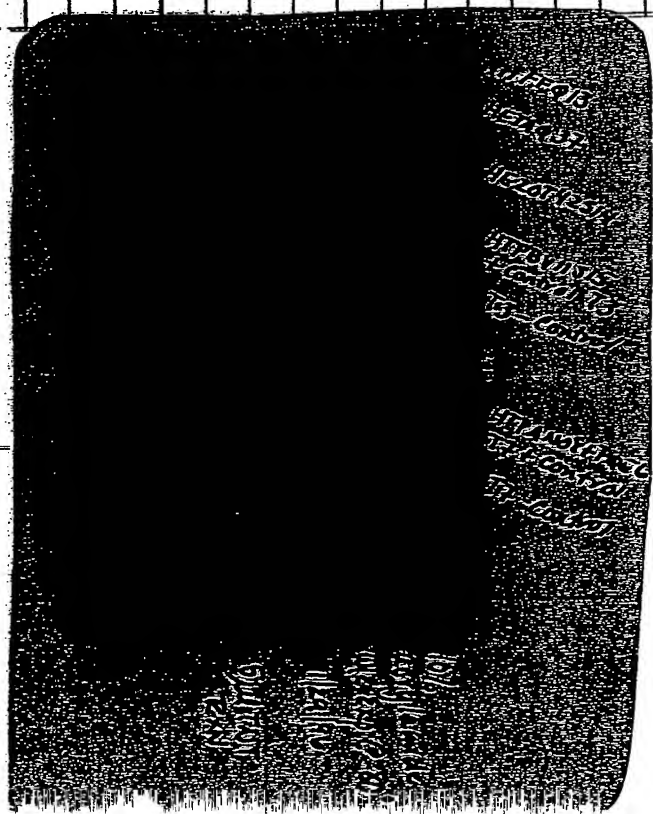
1/26/96



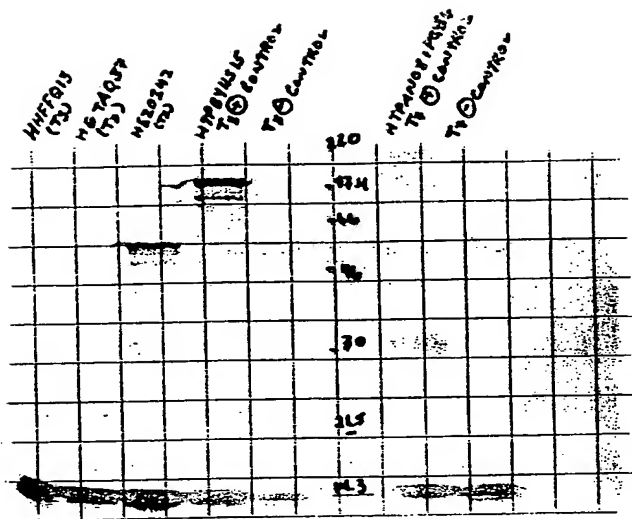
Put on film longer.

1/29/96

Carrie Developed TNT



1/26/96

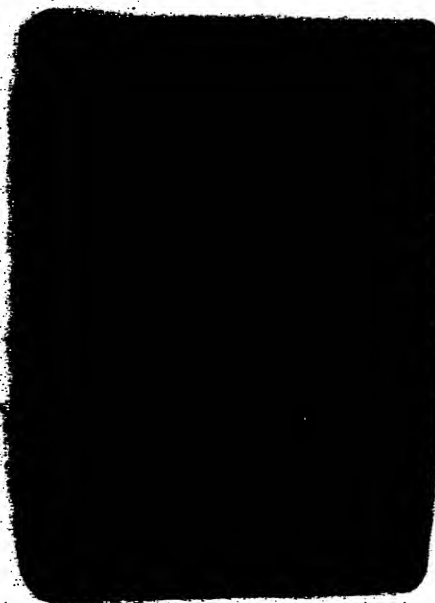


42/96
0.1% Exp
2.5% Acrylamide
Gel

Put on film longer.

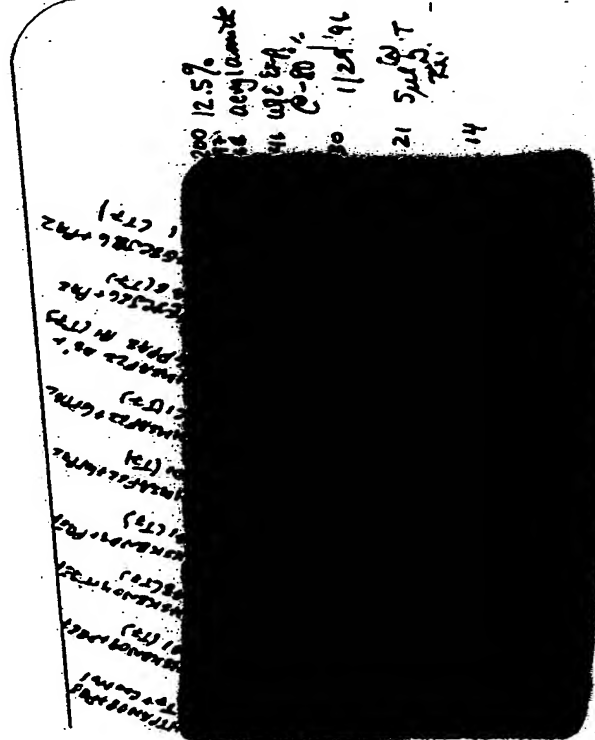
Carrie Developed TNT

1/29/96



10%
acrylamide
w/ 0.1% C-80
1/29/96
SUTUT
ZM

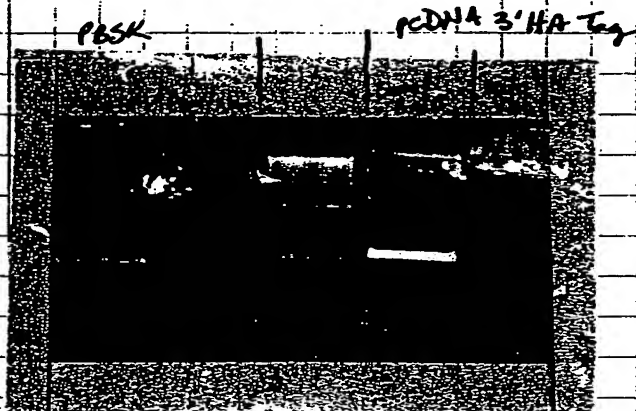
HTP913
HETA937
HETA937
HTP913
+ Control T3
T3 - Control
HTP913 + Control
T3 + Control
T3 - Control



100 12.5%
0.1% acrylamide
0.1% C-80
1/29/96
21 5/25/96
14

(2.1) 1
24.1 7.5%
(2.1) 9.1
24.1 7.5%
(2.1) 11.1
24.1 7.5%
(2.1) 13.1
24.1 7.5%
(2.1) 15.1
24.1 7.5%
(2.1) 17.1
24.1 7.5%
(2.1) 19.1
24.1 7.5%
(2.1) 21.1
24.1 7.5%

1/26/96

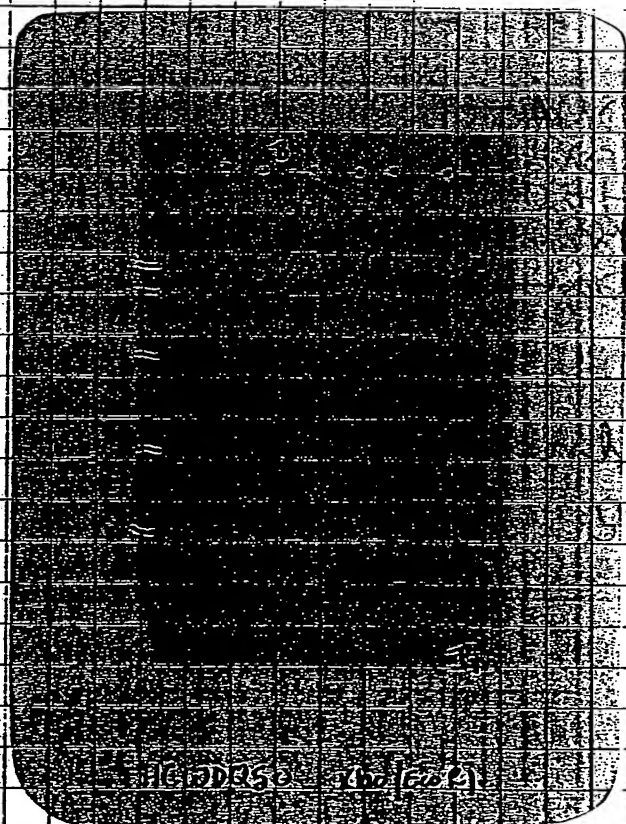
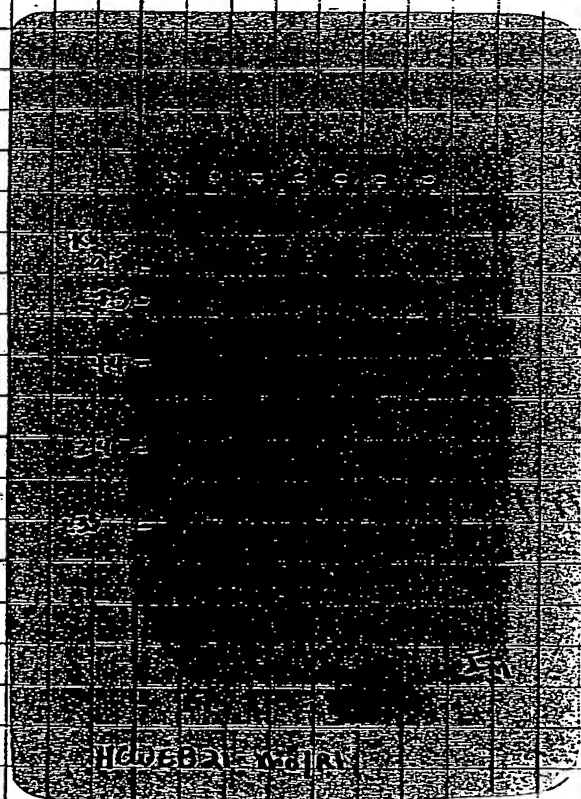


Store gel slices
at -20°C
till Return
from Vacation

Vacation - 1/29/96 → 2/2/96

1/29/96

Carrie -
Develop Northern



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